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|---|---|---|
| NEWS 1 | | Web Page URLs for STN Seminar Schedule - N. America |
| NEWS 2 | | "Ask CAS" for self-help around the clock |
| NEWS 3 | Jun 03 | New e-mail delivery for search results now available |
| NEWS 4 | Aug 08 | PHARMAMarketLetter(PHARMAML) - new on STN |
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| NEWS 6 | Aug 26 | Sequence searching in REGISTRY enhanced |
| NEWS 7 | Sep 03 | JAPIO has been reloaded and enhanced |
| NEWS 8 | Sep 16 | Experimental properties added to the REGISTRY file |
| NEWS 9 | Sep 16 | CA Section Thesaurus available in CAPLUS and CA |
| NEWS 10 | Oct 01 | CASREACT Enriched with Reactions from 1907 to 1985 |
| NEWS 11 | Oct 24 | BEILSTEIN adds new search fields |
| NEWS 12 | Oct 24 | Nutraceuticals International (NUTRACEUT) now available on STN |
| NEWS 13 | Nov 18 | DKILIT has been renamed APOLLIT |
| NEWS 14 | Nov 25 | More calculated properties added to REGISTRY |
| NEWS 15 | Dec 04 | CSA files on STN |
| NEWS 16 | Dec 17 | PCTFULL now covers WP/PCT Applications from 1978 to date |
| NEWS 17 | Dec 17 | TOXCENTER enhanced with additional content |
| NEWS 18 | Dec 17 | Adis Clinical Trials Insight now available on STN |
| NEWS 19 | Jan 29 | Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC |
| NEWS 20 | Feb 13 | CANCERLIT is no longer being updated |
| NEWS 21 | Feb 24 | METADEX enhancements |
| NEWS 22 | Feb 24 | PCTGEN now available on STN |
| NEWS 23 | Feb 24 | TEMA now available on STN |
| NEWS 24 | Feb 26 | NTIS now allows simultaneous left and right truncation |
| NEWS 25 | Feb 26 | PCTFULL now contains images |
| NEWS 26 | Mar 04 | SDI PACKAGE for monthly delivery of multifile SDI results |
| NEWS 27 | Mar 20 | EVENTLINE will be removed from STN |
| NEWS 28 | Mar 24 | PATDPAFULL now available on STN |
| NEWS 29 | Mar 24 | Additional information for trade-named substances without structures available in REGISTRY |
| NEWS 30 | Apr 11 | Display formats in DGENE enhanced |
| NEWS 31 | Apr 14 | MEDLINE Reload |
| NEWS 32 | Apr 17 | Polymer searching in REGISTRY enhanced |
| NEWS 33 | Jun 13 | Indexing from 1947 to 1956 added to records in CA/CAPLUS |
| NEWS 34 | Apr 21 | New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX |
| NEWS 35 | Apr 28 | RDISCLOSURE now available on STN |
| NEWS 36 | May 05 | Pharmacokinetic information and systematic chemical names added to PHAR |
| NEWS 37 | May 15 | MEDLINE file segment of TOXCENTER reloaded |
| NEWS 38 | May 15 | Supporter information for ENCOMPPAT and ENCOMPLIT updated |
| NEWS 39 | May 16 | CHEMREACT will be removed from STN |
| NEWS 40 | May 19 | Simultaneous left and right truncation added to WSCA |
| NEWS 41 | May 19 | RAPRA enhanced with new search field, simultaneous left and |

right truncation
NEWS 42 Jun 06 Simultaneous left and right truncation added to CBNB
NEWS 43 Jun 06 PASCAL enhanced with additional data
NEWS 44 Jun 20 2003 edition of the FSTA Thesaurus is now available

NEWS EXPRESS April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT
MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
NEWS HOURS STN Operating Hours Plus Help Desk Availability
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FILE 'HOME' ENTERED AT 09:58:35 ON 24 JUN 2003

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=> " botulinum toxin"
L1 4606 " BOTULINUM TOXIN"

=> expression and L1
L2 164 EXPRESSION AND L1

=> soluble and L2
L3 8 SOLUBLE AND L2

=> T7 (w) promoter
L4 2643 T7 (W) PROMOTER

=> L4 and l3
L5 0 L4 AND L3

=> T7lac and L3
L6 0 T7LAC AND L3

=> BL21 AND l3
L7 0 BL21 AND L3

```
=> WEAK (W0 PROMOTER
MISSING OPERATOR 'WEAK (W0'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> "weak promoter"
L8          457 "WEAK PROMOTER"

=> L8 and L3
L9            0 L8 AND L3

=> polys (w) gene and L3
L10           0 POLYS (W) GENE AND L3

=> D L3 IBIB TI SO AU ABS1-8
'ABS1-8' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
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REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):IBIB TI SO AU ABS 1-8
'1-8' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):D IBIB
'D' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):D L3 IBIB TI SO AU ABS
'D' IS NOT A VALID FORMAT
'L9' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):  1-8
'1-8' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
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L3   ANSWER 1 OF 8  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2003:409169  CAPLUS
DOCUMENT NUMBER:          138:380506
TITLE:                    Genes that are differentially expressed during
                          erythropoiesis and their diagnostic and therapeutic
                          uses
INVENTOR(S):              Brissette, William H.; Neote, Kuldeep S.; Zagouras,
                          Panayiotis; Zenke, Martin; Lemke, Britt; Hacker,
                          Christine
PATENT ASSIGNEE(S):       Pfizer Products Inc., USA; Max-Delbruck-Centre for
                          Molecular Medicine
SOURCE:                   PCT Int. Appl., 285 pp.
```

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2003038130 | A2 | 20030508 | WO 2002-XA34888 | 20021031 |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | |
| WO 2003038130 | A2 | 20030508 | WO 2002-US34888 | 20021031 |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | |

PRIORITY APPLN. INFO.: US 2001-335048P P 20011031
US 2001-335183P P 20011102
WO 2002-US34888 A 20021031

=> D L3 IBIB TI SO AU ABS 2-8

L3 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:556182 CAPLUS
DOCUMENT NUMBER: 138:182902
TITLE: **Expression** and purification of catalytically active, non-toxic endopeptidase derivatives of Clostridium **botulinum toxin** type A
AUTHOR(S): Chaddock, John A.; Herbert, Michael H.; Ling, Roger J.; Alexander, Frances C. G.; Fooks, Sarah J.; Revell, Dean F.; Quinn, Conrad P.; Shone, Clifford C.; Foster, Keith A.
CORPORATE SOURCE: Centre for Applied Microbiology and Research, Wiltshire, SP4 0JG, UK
SOURCE: Protein Expression and Purification (2002), 25(2), 219-228
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
TI **Expression** and purification of catalytically active, non-toxic

endopeptidase derivatives of Clostridium **botulinum toxin** type A
SO Protein Expression and Purification (2002), 25(2), 219-228
CODEN: PEXPEJ; ISSN: 1046-5928
AU Chaddock, John A.; Herbert, Michael H.; Ling, Roger J.; Alexander, Frances C. G.; Fooks, Sarah J.; Revell, Dean F.; Quinn, Conrad P.; Shone, Clifford C.; Foster, Keith A.
AB Clostridium botulinum neurotoxin type A is a potently toxic protein of 150 kDa with specific endopeptidase activity for the SNARE protein SNAP-25. Proteolytic cleavage of BoNT/A with trypsin leads to removal of the C-terminal domain responsible for neuronal cell binding. Removal of this domain result in a catalytically active, non-cell-binding deriv. termed LHN/A. We have developed a purifn. scheme to prep. LHN/A essentially free of contaminating BoNT/A. LHN/A prepd. by this scheme retains full enzymic activity, is stable in soln., and is of low toxicity as demonstrated in a mouse toxicity assay. In addn., LHN/A has minimal effect on release of neurotransmitter from a primary cell culture model. Both the mouse bioassay and in vitro release assay suggest BoNT/A is present at less than 1 in 106 mols. of LHN/A. This represents a significant improvement on previously reported figures for LHN/A, and also the light chain domain, previously purified from BoNT/A. To complement the prepn. of LHN/A from holotoxin, DNA encoding LHN/A has been introduced into Escherichia coli to facilitate **expression** of recombinant product. **Expression** and purifn. parameters have been developed to enable isolation of **sol**., stable endopeptidase with a toxicity profile enhanced on that of LHN/A purified from BoNT/A. The recombinant-derived material has been used to prep. antisera that neutralize a BoNT/A challenge. The prodn. of essentially BoNT/A-free LHN/A by two different methods and the possibilities for exploitation are discussed.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:549062 CAPLUS
DOCUMENT NUMBER: 135:176585
TITLE: The role of zinc binding in the biological activity of **botulinum toxin**
AUTHOR(S): Simpson, Lance L.; Maksymowych, Andrew B.; Hao, Sheryl
CORPORATE SOURCE: Department of Medicine and Biochemistry and Molecular Pharmacology, Jefferson Medical College, Philadelphia, PA, 19107, USA
SOURCE: Journal of Biological Chemistry (2001), 276(29), 27034-27041
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

TI The role of zinc binding in the biological activity of **botulinum toxin**
SO Journal of Biological Chemistry (2001), 276(29), 27034-27041
CODEN: JBCHA3; ISSN: 0021-9258
AU Simpson, Lance L.; Maksymowych, Andrew B.; Hao, Sheryl
AB **Botulinum toxin** is a zinc-dependent endoprotease that acts on vulnerable cells to cleave polypeptides that are essential for exocytosis. To exert this poisoning effect, the toxin must proceed through a complex sequence of events that involves binding, productive internalization, and intracellular **expression** of catalytic activity. Results presented in this study show that **sol.** chelators rapidly strip Zn2+ from its binding site in **botulinum toxin**, and this stripping of cation results in the loss of catalytic activity in cell-free or broken cell prepns. Stripped toxin is still active against intact neuromuscular junctions, presumably because internalized toxin binds cytosolic Zn2+. In contrast to **sol.** chelators, immobilized chelators have no effect on bound Zn2+, nor do they alter toxin activity. The latter finding is because of the fact that the spontaneous loss of Zn2+ from its coordination site in **botulinum toxin** is relatively slow. When exogenous Zn2+ is added to toxin that has been stripped by **sol.** chelators, the mol. rebinds cation and regains catalytic and neuromuscular blocking activity. Exogenous Zn2+ can restore toxin activity either when the toxin is free in soln. on the cell exterior or when it has been internalized and is in the cytosol. The fact that stripped toxin can reach the cytosol means that the loss of bound Zn2+ does not produce conformational changes that block internalization. Similarly, the fact that stripped toxin in the cytosol can be reactivated by ambient Zn2+ or exogenous Zn2+ means that productive internalization does not produce conformational changes that block rebinding of cation.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:524213 CAPLUS
DOCUMENT NUMBER: 131:269910
TITLE: Tetanus toxin blocks the exocytosis of synaptic vesicles clustered at synapses but not of synaptic vesicles in isolated axons
AUTHOR(S): Verderio, Claudia; Coco, Silvia; Bacci, Alberto; Rossetto, Ornella; De Camilli, Pietro; Montecucco, Cesare; Matteoli, Michela
CORPORATE SOURCE: Department of Medical Pharmacology, Consiglio Nazionale delle Ricerche Cellular and Molecular Pharmacology and B. Ceccarelli Centers, Milan, 20129, Italy
SOURCE: Journal of Neuroscience (1999), 19(16), 6723-6732
CODEN: JNRSDS; ISSN: 0270-6474
PUBLISHER: Society for Neuroscience
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Tetanus toxin blocks the exocytosis of synaptic vesicles clustered at synapses but not of synaptic vesicles in isolated axons
SO Journal of Neuroscience (1999), 19(16), 6723-6732
CODEN: JNRSDS; ISSN: 0270-6474

AU Verderio, Claudia; Coco, Silvia; Bacci, Alberto; Rossetto, Ornella; De Camilli, Pietro; Montecucco, Cesare; Matteoli, Michela

AB Recycling synaptic vesicles are already present in isolated axons of developing neurons (Matteoli et al., 1992; Zakharenko et al., 1999). This vesicle recycling is distinct from the vesicular traffic implicated in axon outgrowth. Formation of synaptic contacts coincides with a clustering of synaptic vesicles at the contact site and with a downregulation of their basal rate of exo-endocytosis (Kraszewski et al., 1995; Coco et al., 1998). We report here that tetanus toxin-mediated cleavage of synaptobrevin/vesicle-assocd. membrane protein (VAMP2), previously shown not to affect axon outgrowth, also does not inhibit synaptic vesicle exocytosis in isolated axons, despite its potent blocking effect on their exocytosis at synapses. This differential effect of tetanus toxin could be seen even on different branches of a same neuron. In contrast, **botulinum toxins** A and E [which cleave synaptosome-assocd. protein of 25 kDa. (SNAP-25)] and F (which cleaves synaptobrevin/VAMP1 and 2) blocked synaptic vesicle exocytosis both in isolated axons and at synapses, strongly suggesting that this process is dependent on "classical" synaptic SNAP receptor (SNARE) complexes both before and after synaptogenesis. A tetanus toxin-resistant form of synaptic vesicle recycling, which proceeds in the absence of external stimuli and is sensitive to **botulinum toxin** F, E, and A, persists at mature synapses. These data suggest the involvement of a tetanus toxin-resistant, but botulinum F-sensitive, isoform of synaptobrevin/VAMP in synaptic vesicle exocytosis before synapse formation and the partial persistence of this form of exocytosis at mature synaptic contacts.

REFERENCE COUNT: 71 THERE ARE 71 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1995:313910 CAPLUS
DOCUMENT NUMBER: 122:72411
TITLE: Calcitonin gene-related peptide: possible role in formation and maintenance of neuromuscular junctions
AUTHOR(S): Sala, C.; Andreose, J. S.; Fumagalli, G.; Loemo, T.
CORPORATE SOURCE: Dep. Pharm., Univ. Milano, Milano, 20129, Italy
SOURCE: Journal of Neuroscience (1995), 15(1, Pt. 2), 520-8
CODEN: JNRSDS; ISSN: 0270-6474
PUBLISHER: Society for Neuroscience
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Calcitonin gene-related peptide: possible role in formation and maintenance of neuromuscular junctions

SO Journal of Neuroscience (1995), 15(1, Pt. 2), 520-8
CODEN: JNRSDS; ISSN: 0270-6474

AU Sala, C.; Andreose, J. S.; Fumagalli, G.; Loemo, T.

AB The **expression** and content of CGRP and secretogranin II (SgII) in adult rat motor neurons were examd. by in situ hybridization, Northern blot anal., and immunocytochem. Normal motor nerve terminals did not contain detectable CGRP or SGII. Ten to 15 days after a peripheral nerve crush .apprx.80% of the motor nerve terminals reinnervating the soleus ( **SOL**) muscle contained detectable CGRP but no SgII. Thereafter, the percentage of CGRP-pos. terminals declined towards zero. In the spinal cord, CGRP **expression** was higher than normal 1 day after

a sciatic nerve crush and increased during the next few days. No increase in SgII **expression** was obsd. Nerve blocks by tetrodotoxin (TTX) and **botulinum toxin** (BoTX) increased CGRP content and **expression** in motor neurons but had no effect on SgII. After 10 days of BoTX treatment and 33 days of TTX treatment (the longest time points studied), >90% of the motor nerve terminals stained for CGRP. The d. of large dense core vesicles (LDCVs) was also higher than normal in such terminals. Some increase in CGRP content and **expression** occurred in the nontreated side. In a group of rats, the peroneal nerve was stimulated elec. with brief, intermittent pulse trains at 100 Hz. The stimulation was applied below a TTX block that had started 7 or 19 days earlier. One min of such stimulation was sufficient to remove CGRP from most of the terminals. These results show (1) that CGRP is upregulated in motor neurons and accumulate in motor nerve terminals during reinnervation and muscle paralysis by BoTX and TTX, (2) that no detectable changes in **expression** or content of SgII occur in the same conditions, and (3) that nerve stimulation causes CGRP to disappear rapidly from the motor nerve terminals, indicating that CGRP is released by nerve impulse activity. An hypothesis for how CGRP may contribute to the formation and maintenance of neuromuscular junctions is presented.

L3 ANSWER 6 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:502146 BIOSIS
DOCUMENT NUMBER: PREV200200502146
TITLE: **Expression** and purification of catalytically active, non-toxic endopeptidase derivatives of Clostridium **botulinum toxin** type A.
AUTHOR(S): Chaddock, John A. (1); Herbert, Michael H.; Ling, Roger J.; Alexander, Frances C. G.; Fooks, Sarah J.; Revell, Dean F.; Quinn, Conrad P.; Shone, Clifford C.; Foster, Keith A.
CORPORATE SOURCE: (1) Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG: john.chaddock@camr.org.uk UK
SOURCE: Protein Expression and Purification, (July, 2002) Vol. 25, No. 2, pp. 219-228. http://www.academicpress.com/pep. print.
ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
TI **Expression** and purification of catalytically active, non-toxic endopeptidase derivatives of Clostridium **botulinum toxin** type A.
SO Protein Expression and Purification, (July, 2002) Vol. 25, No. 2, pp. 219-228. http://www.academicpress.com/pep. print.
ISSN: 1046-5928.
AU Chaddock, John A. (1); Herbert, Michael H.; Ling, Roger J.; Alexander, Frances C. G.; Fooks, Sarah J.; Revell, Dean F.; Quinn, Conrad P.; Shone, Clifford C.; Foster, Keith A.
AB Clostridium botulinum neurotoxin type A is a potently toxic protein of 150 kDa with specific endopeptidase activity for the SNARE protein SNAP-25. Proteolytic cleavage of BoNT/A with trypsin leads to removal of the C-terminal domain responsible for neuronal cell binding. Removal of this

domain result in a catalytically active, non-cell-binding derivative termed LHN/A. We have developed a purification scheme to prepare LHN/A essentially free of contaminating BoNT/A. LHN/A prepared by this scheme retains full enzymatic activity, is stable in solution, and is of low toxicity as demonstrated in a mouse toxicity assay. In addition, LHN/A has minimal effect on release of neurotransmitter from a primary cell culture model. Both the mouse bioassay and in vitro release assay suggest BoNT/A is present at less than 1 in 106 molecules of LHN/A. This represents a significant improvement on previously reported figures for LHN/A, and also the light chain domain, previously purified from BoNT/A. To complement the preparation of LHN/A from holotoxin, DNA encoding LHN/A has been introduced into Escherichia coli to facilitate **expression** of recombinant product. **Expression** and purification parameters have been developed to enable isolation of **soluble**, stable endopeptidase with a toxicity profile enhanced on that of LHN/A purified from BoNT/A. The recombinant-derived material has been used to prepare antisera that neutralise a BoNT/A challenge. The production of essentially BoNT/A-free LHN/A by two different methods and the possibilities for exploitation are discussed.

L3 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:323357 BIOSIS
DOCUMENT NUMBER: PREV200200323357
TITLE: Changes in SNARE protein immunoreactivity in mouse muscle following injection of **botulinum toxin** correlate with signs of paralysis and recovery of function.
AUTHOR(S): Whelchel, Dee D. (1); Brooks, Paula M. (1); Coffield, Julie A. (1)
CORPORATE SOURCE: (1) Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602 USA
SOURCE: FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A185-A186. http://www.fasebj.org/. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English
TI Changes in SNARE protein immunoreactivity in mouse muscle following injection of **botulinum toxin** correlate with signs of paralysis and recovery of function.
SO FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A185-A186. http://www.fasebj.org/. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638.
AU Whelchel, Dee D. (1); Brooks, Paula M. (1); Coffield, Julie A. (1)
AB Botulinum neurotoxin induces muscle paralysis through its presynaptic action at the neuromuscular junction by selectively cleaving specific SNARE proteins. This study examined whether in vivo changes in SNARE protein could be correlated with signs of paralysis and recovery. Mice were injected in the right gastrocnemius muscle with toxin type A (2.5pg) and monitored for signs of paralysis. Within 24-48hrs, mice showed evidence of paralysis in injected limbs, with peak paralysis occurring

between 48-72 hrs. Five to seven days postinjection (PI), signs of paralysis began to abate. At 7 days PI, mice were sacrificed, muscles collected, and SNARE protein content examined using western blot techniques. Syntaxin, SNAP-25 and VAMP content from injected muscles were analyzed and compared to muscles from saline injected limb; SNAP-25 cleavage was also analyzed. Preliminary results from toxin-treated gastrocnemius muscles revealed the SNAP-25 cleavage product (24 kDa). Further, increases in both full length SNAP-25 and VAMP II immunoreactivity were evident in the toxin-injected gastrocnemius and soleus muscles compared to saline injected controls. These findings suggest that 1) **botulinum toxin**-induced paralysis in mice is correlated with substrate cleavage in vivo and 2) recovery of function following toxin injection can be correlated with increases in SNARE protein content suggesting either an upregulation of these proteins in existing nerve endings or sprouting of new nerve endings.

L3 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:406058 BIOSIS
DOCUMENT NUMBER: PREV200100406058
TITLE: The role of zinc binding in the biological activity of **botulinum toxin**.
AUTHOR(S): Simpson, Lance L. (1); Maksymowych, Andrew B.; Hao, Sheryl
CORPORATE SOURCE: (1) Departments of Medicine and Biochemistry and Molecular Pharmacology, Jefferson Medical College, Philadelphia, PA, 19107: lance.simpson@mail.tju.edu USA
SOURCE: Journal of Biological Chemistry, (July 20, 2001) Vol. 276, No. 29, pp. 27034-27041. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI The role of zinc binding in the biological activity of **botulinum toxin**.
SO Journal of Biological Chemistry, (July 20, 2001) Vol. 276, No. 29, pp. 27034-27041. print.
ISSN: 0021-9258.
AU Simpson, Lance L. (1); Maksymowych, Andrew B.; Hao, Sheryl
AB **Botulinum toxin** is a zinc-dependent endoprotease that acts on vulnerable cells to cleave polypeptides that are essential for exocytosis. To exert this poisoning effect, the toxin must proceed through a complex sequence of events that involves binding, productive internalization, and intracellular **expression** of catalytic activity. Results presented in this study show that **soluble** chelators rapidly strip Zn2+ from its binding site in **botulinum toxin**, and this stripping of cation results in the loss of catalytic activity in cell-free or broken cell preparations. Stripped toxin is still active against intact neuromuscular junctions, presumably because internalized toxin binds cytosolic Zn2+. In contrast to **soluble** chelators, immobilized chelators have no effect on bound Zn2+, nor do they alter toxin activity. The latter finding is because of the fact that the spontaneous loss of Zn2+ from its coordination site in **botulinum toxin** is relatively slow. When exogenous Zn2+ is added to toxin that has been stripped by **soluble** chelators, the molecule rebinds cation and regains catalytic and neuromuscular blocking activity. Exogenous Zn2+ can restore toxin activity either when the toxin is free in solution on the cell exterior or when it has been internalized and is in the cytosol. The fact that stripped toxin can reach the cytosol means that the loss of bound Zn2+ does not produce

conformational changes that block internalization. Similarly, the fact that stripped toxin in the cytosol can be reactivated by ambient Zn2+ or exogenous Zn2+ means that productive internalization does not produce conformational changes that block rebinding of cation.

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF

FILE COVERS 1947 - 29 Aug 2001 VOL 135 ISS 10
FILE LAST UPDATED: 28 Aug 2001 (20010828/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

This file supports REG1stRY for direct browsing and searching of all substance data from the REGISTRY file. Enter HELP FIRST for more information.

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The CA Lexicon is now available in the Controlled Term (/CT) field. Enter HELP LEXICON for full details.



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=> S (RECOMBINANT (5A) CLOSTIDIUM (5A) BOTULINUM (5A) TOXIN (5A) A)
        120844 RECOMBINANT
          5614 RECOMBINANTS
        123975 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
            16 CLOSTIDIUM
          3670 BOTULINUM
             1 BOTULINUMS
          3670 BOTULINUM
                 (BOTULINUM OR BOTULINUMS)
         57622 TOXIN
         52805 TOXINS
         83173 TOXIN
                 (TOXIN OR TOXINS)
      14698207 A
L1           0 (RECOMBINANT (5A) CLOSTIDIUM (5A) BOTULINUM (5A) TOXIN (5A) A)

=> S (RECOMBINANT (W) CLOSTIDIUM (W) BOTULINUM (W) TOXIN (W) A)
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          5614 RECOMBINANTS
        123975 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
            16 CLOSTIDIUM
          3670 BOTULINUM
             1 BOTULINUMS
          3670 BOTULINUM
                 (BOTULINUM OR BOTULINUMS)
         57622 TOXIN
         52805 TOXINS
         83173 TOXIN
                 (TOXIN OR TOXINS)
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      14698207 A
L2           0 (RECOMBINANT (W) CLOSTIDIUM (W) BOTULINUM (W) TOXIN (W) A)

=> S (RECOMBINANT (S) CLOSTIDIUM (S) BOTULINUM (S) TOXIN (S) A)
         120844 RECOMBINANT
           5614 RECOMBINANTS
         123975 RECOMBINANT
                  (RECOMBINANT OR RECOMBINANTS)
             16 CLOSTIDIUM
           3670 BOTULINUM
              1 BOTULINUMS
           3670 BOTULINUM
                  (BOTULINUM OR BOTULINUMS)
          57622 TOXIN
          52805 TOXINS
          83173 TOXIN
                  (TOXIN OR TOXINS)
      14698207 A
L3           0 (RECOMBINANT (S) CLOSTIDIUM (S) BOTULINUM (S) TOXIN (S) A)

=> S (CLOSTIDIUM (S) BOTULINUM (S) TOXIN (S) A)
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           3670 BOTULINUM
              1 BOTULINUMS
           3670 BOTULINUM
                  (BOTULINUM OR BOTULINUMS)
          57622 TOXIN
          52805 TOXINS
          83173 TOXIN
                  (TOXIN OR TOXINS)
      14698207 A
L4           0 (CLOSTIDIUM (S) BOTULINUM (S) TOXIN (S) A)

=> S (CLOSTIDIUM (W) BOTULINUM (W) TOXIN (W) A)
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           3670 BOTULINUM
              1 BOTULINUMS
           3670 BOTULINUM
                  (BOTULINUM OR BOTULINUMS)
          57622 TOXIN
          52805 TOXINS
          83173 TOXIN
                  (TOXIN OR TOXINS)
      14698207 A
L5           0 (CLOSTIDIUM (W) BOTULINUM (W) TOXIN (W) A)

=> S (CLOSTRIDIUM (W) BOTULINUM (W) TOXIN (W) A)
          18538 CLOSTRIDIUM
              2 CLOSTRIDIUMS
           1157 CLOSTRIDIA
          18910 CLOSTRIDIUM
                  (CLOSTRIDIUM OR CLOSTRIDIUMS OR CLOSTRIDIA)
           3670 BOTULINUM
              1 BOTULINUMS
           3670 BOTULINUM
                  (BOTULINUM OR BOTULINUMS)
          57622 TOXIN
          52805 TOXINS
          83173 TOXIN
                  (TOXIN OR TOXINS)
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      14698207 A
L6           71 (CLOSTRIDIUM (W) BOTULINUM (W) TOXIN (W) A)

=> S L6 AND (RECOMBINANT L6)
MISSING OPERATOR COMBINANT L6
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> S L6 AND (RECOMBINANT AND L6)
         120844 RECOMBINANT
           5614 RECOMBINANTS
         123975 RECOMBINANT
                  (RECOMBINANT OR RECOMBINANTS)
L7            0 L6 AND (RECOMBINANT AND L6)

=> S L6 AND (RECOMBINANT CLOSTRIDIUM BOTULINUM TOXIN A)
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           5614 RECOMBINANTS
         123975 RECOMBINANT
                  (RECOMBINANT OR RECOMBINANTS)
          18538 CLOSTRIDIUM
              2 CLOSTRIDIUMS
           1157 CLOSTRIDIA
          18910 CLOSTRIDIUM
                  (CLOSTRIDIUM OR CLOSTRIDIUMS OR CLOSTRIDIA)
           3670 BOTULINUM
              1 BOTULINUMS
           3670 BOTULINUM
                  (BOTULINUM OR BOTULINUMS)
          57622 TOXIN
          52805 TOXINS
          83173 TOXIN
                  (TOXIN OR TOXINS)
      14698207 A
              0 RECOMBINANT CLOSTRIDIUM BOTULINUM TOXIN A
                  (RECOMBINANT(W)CLOSTRIDIUM(W)BOTULINUM(W)TOXIN(W)A)
L8            0 L6 AND (RECOMBINANT CLOSTRIDIUM BOTULINUM TOXIN A)

=> S L6 AND (RECOMBINANT CLOSTRIDIUM BOTULINUM TOXIN )
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           5614 RECOMBINANTS
         123975 RECOMBINANT
                  (RECOMBINANT OR RECOMBINANTS)
          18538 CLOSTRIDIUM
              2 CLOSTRIDIUMS
           1157 CLOSTRIDIA
          18910 CLOSTRIDIUM
                  (CLOSTRIDIUM OR CLOSTRIDIUMS OR CLOSTRIDIA)
           3670 BOTULINUM
              1 BOTULINUMS
           3670 BOTULINUM
                  (BOTULINUM OR BOTULINUMS)
          57622 TOXIN
          52805 TOXINS
          83173 TOXIN
                  (TOXIN OR TOXINS)
              0 RECOMBINANT CLOSTRIDIUM BOTULINUM TOXIN
                  (RECOMBINANT(W)CLOSTRIDIUM(W)BOTULINUM(W)TOXIN)
L9            0 L6 AND (RECOMBINANT CLOSTRIDIUM BOTULINUM TOXIN )
```

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=> S (RECOMBINANT (W) CLOSTRIDIUM (W) BOTULINUM (W) TOXIN)
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          5614 RECOMBINANTS
        123975 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
         18538 CLOSTRIDIUM
             2 CLOSTRIDIUMS
          1157 CLOSTRIDIA
         18910 CLOSTRIDIUM
                 (CLOSTRIDIUM OR CLOSTRIDIUMS OR CLOSTRIDIA)
          3670 BOTULINUM
             1 BOTULINUMS
          3670 BOTULINUM
                 (BOTULINUM OR BOTULINUMS)
         57622 TOXIN
         52805 TOXINS
         83173 TOXIN
                 (TOXIN OR TOXINS)
L10          0 (RECOMBINANT (W) CLOSTRIDIUM (W) BOTULINUM (W) TOXIN)

=> S (RECOMBINANT (5A) CLOSTRIDIUM (5A) BOTULINUM (5A) TOXIN)
        120844 RECOMBINANT
          5614 RECOMBINANTS
        123975 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
         18538 CLOSTRIDIUM
             2 CLOSTRIDIUMS
          1157 CLOSTRIDIA
         18910 CLOSTRIDIUM
                 (CLOSTRIDIUM OR CLOSTRIDIUMS OR CLOSTRIDIA)
          3670 BOTULINUM
             1 BOTULINUMS
          3670 BOTULINUM
                 (BOTULINUM OR BOTULINUMS)
         57622 TOXIN
         52805 TOXINS
         83173 TOXIN
                 (TOXIN OR TOXINS)
L11          5 (RECOMBINANT (5A) CLOSTRIDIUM (5A) BOTULINUM (5A) TOXIN)

=> S (RECOMBINANT (S) CLOSTRIDIUM (S) BOTULINUM (S) TOXIN)
        120844 RECOMBINANT
          5614 RECOMBINANTS
        123975 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
         18538 CLOSTRIDIUM
             2 CLOSTRIDIUMS
          1157 CLOSTRIDIA
         18910 CLOSTRIDIUM
                 (CLOSTRIDIUM OR CLOSTRIDIUMS OR CLOSTRIDIA)
          3670 BOTULINUM
             1 BOTULINUMS
          3670 BOTULINUM
                 (BOTULINUM OR BOTULINUMS)
         57622 TOXIN
         52805 TOXINS
         83173 TOXIN
                 (TOXIN OR TOXINS)
L12         11 (RECOMBINANT (S) CLOSTRIDIUM (S) BOTULINUM (S) TOXIN)
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=> DIS L12 1 IBIB ABS
THE ESTIMATED COST FOR THIS REQUEST IS 2.17 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L12 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:401109 CAPLUS
TITLE: Regulation by Rho family GTPases of IL-1 receptor induced signaling: C3-like chimeric toxin and Clostridium difficile toxin B inhibit signaling pathways involved in IL-2 gene expression
AUTHOR(S): Dreikhausen, Ursula; Varga, Georg; Hofmann, Fred; Barth, Holger; Aktories, Klaus; Resch, Klaus; Szamel, Marta
CORPORATE SOURCE: Institute of Pharmacology, Medical School Hannover, Hannover, Germany
SOURCE: Eur. J. Immunol. (2001), 31(5), 1610-1619
CODEN: EJIMAF; ISSN: 0014-2980
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study the participation of Rho family GTPases in the regulation of IL-1-activated protein kinase cascades controlling IL-2 synthesis was investigated in murine EL-4 thymoma cells. The **recombinant** C3-like chimeric **toxin**, which consists of the C3 **toxin** of **Clostridium** limosum and the N-terminal part of **Clostridium botulinum** C2 **toxin** (C2IN-C3) interacting with the C2II binding subunit to facilitate uptake into cells, and selectively inactivates Rho A by ADP-ribosylation, prevented IL-1-stimulated activation of Jun-NH2-terminal-kinases (JNK) and p38 mitogen-activated-protein kinases (MAPK). UDP-monoglucosylation and concomitant inactivation of Rho A and of Rac-2 by Clostridium difficile toxin B also inhibited IL-1-induced activation of JNK and p38 MAPK, but addnl. inhibited activation of the extracellular-regulated-kinase pathway and DNA binding of the transcription factor NF.kappa.B. Accordingly, pre-treatment of cells with C2IN-C3 fusion toxin only decreased IL-1-stimulated IL-2 synthesis by 50%, while in C. difficile toxin B-treated cells IL-1-induced IL-2 secretion was reduced by 90%. These results imply that together with Rho A an addnl. member of the Rho family G proteins, i.e. Rac-2, is critically involved as an upstream regulator in IL-1-induced activation of different MAPK, stress-activated protein kinases, and in NF.kappa.B activation controlling IL-2 gene expression in response to IL-1, acting in close proximity to the IL-1-receptor complex.
REFERENCE COUNT: 36
REFERENCE(S): (2) Aspenstrom, P; Curr Opin Cell Biol 1999, V11, P95 CAPLUS
(4) Baeuerle, P; Annu Rev Immunol 1994, V12, P141 CAPLUS
(5) Barth, H; Infection and Immunity 1998, V66, P1364 CAPLUS
(7) Cantrell, D; Ann Rev Immunol 1996, V14, P259 CAPLUS
(8) Coso, O; Cell 1995, V81, P1137 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> DIS L12 1- IBIB ABS
YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):Y

THE ESTIMATED COST FOR THIS REQUEST IS 23.91 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L12 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:401109 CAPLUS
TITLE: Regulation by Rho family GTPases of IL-1 receptor induced signaling: C3-like chimeric toxin and Clostridium difficile toxin B inhibit signaling pathways involved in IL-2 gene expression
AUTHOR(S): Dreikhausen, Ursula; Varga, Georg; Hofmann, Fred; Barth, Holger; Aktories, Klaus; Resch, Klaus; Szamel, Marta
CORPORATE SOURCE: Institute of Pharmacology, Medical School Hannover, Hannover, Germany
SOURCE: Eur. J. Immunol. (2001), 31(5), 1610-1619
CODEN: EJIMAF; ISSN: 0014-2980
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study the participation of Rho family GTPases in the regulation of IL-1-activated protein kinase cascades controlling IL-2 synthesis was investigated in murine EL-4 thymoma cells. The **recombinant** C3-like chimeric **toxin**, which consists of the C3 **toxin** of **Clostridium** limosum and the N-terminal part of **Clostridium botulinum** C2 **toxin** (C2IN-C3) interacting with the C2II binding subunit to facilitate uptake into cells, and selectively inactivates Rho A by ADP-ribosylation, prevented IL-1-stimulated activation of Jun-NH2-terminal-kinases (JNK) and p38 mitogen-activated-protein kinases (MAPK). UDP-monoglucosylation and concomitant inactivation of Rho A and of Rac-2 by Clostridium difficile toxin B also inhibited IL-1-induced activation of JNK and p38 MAPK, but addnl. inhibited activation of the extracellular-regulated-kinase pathway and DNA binding of the transcription factor NF.kappa.B. Accordingly, pre-treatment of cells with C2IN-C3 fusion toxin only decreased IL-1-stimulated IL-2 synthesis by 50%, while in C. difficile toxin B-treated cells IL-1-induced IL-2 secretion was reduced by 90%. These results imply that together with Rho A an addnl. member of the Rho family G proteins, i.e. Rac-2, is critically involved as an upstream regulator in IL-1-induced activation of different MAPK, stress-activated protein kinases, and in NF.kappa.B activation controlling IL-2 gene expression in response to IL-1, acting in close proximity to the IL-1-receptor complex.
REFERENCE COUNT: 36
REFERENCE(S): (2) Aspenstrom, P; Curr Opin Cell Biol 1999, V11, P95 CAPLUS
(4) Baeuerle, P; Annu Rev Immunol 1994, V12, P141 CAPLUS
(5) Barth, H; Infection and Immunity 1998, V66, P1364 CAPLUS
(7) Cantrell, D; Ann Rev Immunol 1996, V14, P259 CAPLUS
(8) Coso, O; Cell 1995, V81, P1137 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:227474 CAPLUS
DOCUMENT NUMBER: 135:97336
TITLE: Recombinant derivatives of clostridial neurotoxins as

delivery vehicles for proteins and small organic molecules
AUTHOR(S): Zdanovskaia, Marina V.; Los, Georgyi; Zdanovsky, Alexey G.
CORPORATE SOURCE: Promega Corporation, Madison, WI, 53711-5399, USA
SOURCE: J. Protein Chem. (2000), 19(8), 699-707
CODEN: JPCHD2; ISSN: 0277-8033
PUBLISHER: Kluwer Academic/Plenum Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Clostridial neurotoxins are the most powerful toxins known. Nevertheless, derivs. of these toxins may find broad applications both in science and medicine because of their unique abilities to recognize neurons and deliver small and large mols. into them. In this paper we describe the construction of two types of such derivs. Proteins belonging to the first class were designed to allow direct conjugation with one or few mols. of interest. Proteins belonging to the second class contain biotin residues and therefore could be easily connected to streptavidin loaded with multiple mols. of interest. Only C-terminal regions of neurotoxin heavy chains were incorporated in the structure of recombinant proteins. Nevertheless, recombinant proteins were found to be able to recognize specific neuronal receptors and target model mols. to rat synaptosomes and human neuroblastoma cells.
REFERENCE COUNT: 27
REFERENCE(S): (1) Binz, T; Nucleic Acids Res 1990, V18, P5556 CAPLUS
(2) Bizzini, B; Brain Res 1981, V210, P291 CAPLUS
(4) Dunkley, P; Brain Res 1988, V441, P59 CAPLUS
(6) Eisel, U; EMBO J 1986, V5, P2495 CAPLUS
(7) Figueiredo, D; Exp Neurol 1997, V145, P546 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:908058 CAPLUS
DOCUMENT NUMBER: 134:97731
TITLE: Effective expression of type A botulinic neurotoxin gene fragments in Escherichia coli: immunization with recombinant I and H chains protects against the toxin
AUTHOR(S): Vertiev, Yu. V.; Zdanovsky, A. G.; Borinskaya, S. A.; Martin, T.; Gening, E. L.; Yankovsky, N. K.
CORPORATE SOURCE: N.F. Gamaleya Institute epidemiology and Microbiology, Russian Academy Medical Sciences, Moscow, Russia
SOURCE: Mol. Genet., Mikrobiol. Virusol. (2000), (4), 3-7
CODEN: MGMVDU; ISSN: 0208-0613
PUBLISHER: Meditsina
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB Native **Clostridium botulinum** gene coding for type A neurotoxin has been used to construct **recombinant** derivs. coding sep. for L and H polypeptide chains of the **toxin**. The gene derivs. have been cloned into an expression vector pET28b in E. Coli BL21 (DE3) cells. The recombinant L and H proteins seem to be the major individual proteins after IPTG induction of the recombinant cells. Each of the proteins has been accumulated only in inclusion bodies. The recombinant L chain (but not H chain) has been successfully resolubilized.

Each of the proteins contains six His residues on the N terminus which allows purifn. on Ni-agarose columns with high yield. No toxic effect has been obsd. for both L and H chains after injection of 10 .mu.g of recombinant prepns. purified from inclusion bodies. Moreover, the injection resulted in an increase in the titer of specific antibodies which protected mice from 1 DLM of type A native botulinum neurotoxin. Hence, the recombinant neurotoxin protein derivs. which are present in E. coli inclusion bodies can be a source of material for producing diagnostic and therapeutic sera against type A botulinum neurotoxin.

L12 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:526203 CAPLUS
DOCUMENT NUMBER: 134:264774
TITLE: Cloning, expression and evaluation of a **recombinant** sub-unit vaccine against **Clostridium botulinum** type F **toxin**
AUTHOR(S): Holley, J. L.; Elmore, M.; Mauchline, M.; Minton, N.; Titball, R. W.
CORPORATE SOURCE: CBD Porton Down, Defence Evaluation and Research Agency, Salisbury, Wilts, SP4 0JQ, UK
SOURCE: Vaccine (2000), 19(2-3), 288-297
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A synthetic gene encoding the Hc (binding) domain of Clostridium botulinum neurotoxin F (FHc) was expressed in Escherichia coli fused to maltose binding protein (MBP). The purified MBP-FHc and FHc isolated after removal of MBP were evaluated in mice for their ability to protect against toxin challenge. Balb/c mice developed a protective immune response following administration of either protein via the i.p. or i.m. routes. A comparison of antibody titers and protection following single and multiple vaccinations and the effects of dosage are shown. The long term protection afforded by the vaccines was also investigated. Ten months following vaccination mice were still protected when challenged with 104 MLD50 doses of botulinum toxin F.
REFERENCE COUNT: 18
REFERENCE(S): (4) Byrne, M; Infect Immun 1998, V66, P4817 CAPLUS
(5) Chambers, S; Gene 1988, V68, P139 CAPLUS
(6) Chen, F; Infect Immun 1997, V65, P1626 CAPLUS
(7) Clare, J; Biotechnology 1991, V9, P455 CAPLUS
(8) Clayton, M; Infect Immun 1995, V63, P2738 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:161466 CAPLUS
DOCUMENT NUMBER: 132:204055
TITLE: Production of clostridial toxins with recombinant cells producing rare codon-recognizing tRNAs
INVENTOR(S): Zdanovsky, Alexey G.
PATENT ASSIGNEE(S): Promega Corporation, USA
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000012728 | A1 | 20000309 | WO 1999-US19284 | 19990823 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 6214602 | B1 | 20010410 | US 1998-143634 | 19980828 |
| AU 9956885 | A1 | 20000321 | AU 1999-56885 | 19990823 |

PRIORITY APPLN. INFO.: US 1998-143634 A 19980828
WO 1999-US19284 W 19990823

AB The present invention is directed to methods and compns. useful in the overprodn. of Clostridium toxins and proteins by hosts such as Escherichia coli. The host cell is genetically altered to produce tRNAs which recognize rare codons. These proteins and toxins find use in various medical and veterinary applications, including vaccine prodn., and cosmetic dermatol., as well as treatment of neurol. and other diseases and conditions. Thus, E. coli were transformed with plasmids contg. the ileX, argU and leuW genes and plasmids encoding Clostridium botulinum B, C and E toxins or C3 protein, iota toxin Ia protein of Clostridium perfringens, or tetanus toxin. Relative to wild-type E. coli, increased amts. of enzymically active toxins were produced by these transformants.

REFERENCE COUNT: 7
REFERENCE(S):
(2) Kim; Biotechnology Letters 1998, V20(3), P207 CAPLUS
(3) Komine; J Molecular Biology 1990, V212, P579 CAPLUS
(4) Makoff; Nucleic Acids Research 1989, V17(24), P10191 CAPLUS
(5) Makrides, S; Microbiological Reviews 1996, V60(3), P512 CAPLUS
(6) Nakajima; Cell 1981, V23, P239 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:425504 CAPLUS
DOCUMENT NUMBER: 131:72729
TITLE: Vaccine for Clostridium botulinum neurotoxin
INVENTOR(S): Williams, James A.
PATENT ASSIGNEE(S): Ophidian Pharmaceuticals, Inc., USA
SOURCE: U.S., 140 pp., Cont.-in-part of U.S. Ser. No. 329,154, abandoned.
CODEN: USXXAM

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 9
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 5919665 | A | 19990706 | US 1995-405496 | 19950316 |
| US 5196193 | A | 19930323 | US 1989-429791 | 19891031 |
| US 5601823 | A | 19970211 | US 1993-161907 | 19931202 |
| US 5599539 | A | 19970204 | US 1994-255009 | 19940607 |
| US 5443976 | A | 19950822 | US 1994-275304 | 19940714 |
| US 5904922 | A | 19990518 | US 1995-442000 | 19950516 |
| US 5736139 | A | 19980407 | US 1995-480604 | 19950607 |
| CA 2203504 | AA | 19960502 | CA 1995-2203504 | 19951023 |
| WO 9612802 | A1 | 19960502 | WO 1995-US13737 | 19951023 |
| W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| AU 9539683 | A1 | 19960515 | AU 1995-39683 | 19951023 |
| AU 709586 | B2 | 19990902 | | |
| EP 796326 | A1 | 19970924 | EP 1995-937626 | 19951023 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| BR 9509903 | A | 19971125 | BR 1995-9903 | 19951023 |
| CN 1176658 | A | 19980318 | CN 1995-196424 | 19951023 |
| HU 78048 | A2 | 19990728 | HU 1999-1238 | 19951023 |
| EP 1041149 | A2 | 20001004 | EP 2000-105371 | 19951023 |
| EP 1041149 | A3 | 20010502 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV | | | | |
| ZA 9508990 | A | 19960515 | ZA 1995-8990 | 19951024 |
| FI 9701732 | A | 19970623 | FI 1997-1732 | 19970423 |
| NO 9701868 | A | 19970624 | NO 1997-1868 | 19970423 |
| AU 9948763 | A1 | 19991125 | AU 1999-48763 | 19990916 |

PRIORITY APPLN. INFO.:

| | | |
|---|---|---|
| US 1989-429791 | A2 | 19891031 |
| US 1992-985321 | A2 | 19921204 |
| US 1993-161907 | A2 | 19931202 |
| US 1994-329154 | B2 | 19941024 |
| US 1992-842709 | A2 | 19920226 |
| US 1992-983668 | B1 | 19921201 |
| US 1994-275304 | A3 | 19940714 |
| US 1995-405496 | A2 | 19950316 |
| US 1995-422711 | A2 | 19950414 |
| US 1995-480604 | A | 19950607 |
| AU 1995-39683 | A3 | 19951023 |
| EP 1995-937626 | A3 | 19951023 |
| WO 1995-US13737 | W | 19951023 |

AB The present invention includes **recombinant** proteins derived from **toxins** of **Clostridium botulinum** and **Clostridium** difficile. In particular, sol. **recombinant** fusion proteins comprising **Clostridium botulinum** type A **toxin** proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The sol., endotoxin-free recombinant proteins

are used as immunogens for the prodn. of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.

REFERENCE COUNT: 117
REFERENCE(S): (1) Afrin; Bioconj Chem 1994, V5, P539 CAPLUS
(12) Barroso; Nucl Acids Res 1990, V18, P4004 CAPLUS
(13) Beitle; Biotechnol Prog 1993, V9, P64 CAPLUS
(15) Benson, H; J Immunol 1961, V87, P616 CAPLUS
(21) Carroll; US 5196193 1993 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:263115 CAPLUS
DOCUMENT NUMBER: 128:305019
TITLE: Expression of mouse synaptobrevin (VAMP) gene in E. coli and its cleavage by the Clostridium botulinum type B toxin
AUTHOR(S): Jung, Hyun Ho; Yang, Gi-Hyeok; Rhee, Sang Dal; Yang, Kyu-Hwan
CORPORATE SOURCE: Dep. of Microbiol., Sunmoon Univ., Asan, 336-840, S. Korea
SOURCE: Korean J. Toxicol. (1997), 13(4), 417-421
CODEN: KJTOEA; ISSN: 0258-2368
PUBLISHER: Korean Society of Toxicology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Synaptobrevin is a kind of vesicle assocd. membrane proteins (VAMPs) which plays a secretary role in the neuronal synapse and was recently known as the biochem. target of botulinum neurotoxin type B. The structural gene of synaptobrevin was cloned from mouse brain using RT-PCR technique and was sequenced. The deduced amino acid sequence showed that the synaptobrevin protein from mouse brain is exactly the same with that of the rat brain in the amino acid level. The synaptobrevin gene was subcloned into pET3a vector and expressed in E. coli. The mol. wt. of the recombinant protein was 19 kDa as expected. Moreover, when the **recombinant** synaptobrevin protein was incubated with the native neurotoxin of **Clostridium botulinum** type B, it was cleaved by the **toxin** in a time dependent manner. This implies that the recombinant synaptobrevin protein and the native toxin are reacted in the same way as the native synaptobrevin did in the neuronal cells.

L12 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:163478 CAPLUS
DOCUMENT NUMBER: 128:242882
TITLE: Multivalent vaccine for Clostridium botulinum neurotoxin
INVENTOR(S): Williams, James A.; Thalley, Bruce S.
PATENT ASSIGNEE(S): Ophidian Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 428 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

WO 9808540 A1 19980305 WO 1997-US15394 19970828
W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AU 9742450 A1 19980319 AU 1997-42450 19970828
EP 1105153 A1 20010613 EP 1997-940746 19970828
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
PRIORITY APPLN. INFO.: US 1996-704159 A 19960828
WO 1997-US15394 W 19970828

AB The present invention includes **recombinant** proteins derived from **Clostridium botulinum toxins**. In particular, sol. **recombinant Clostridium botulinum** type A, type B and type E **toxin** proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The sol., endotoxin-free recombinant proteins are used as immunogens for the prodn. of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin. Thus, recombinant C. difficile toxin A and B gene and proteins and C. botulinum type A.apprx.G neurotoxin gene and proteins were prepd. as vaccines.

L12 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:7885 CAPLUS
DOCUMENT NUMBER: 128:162761
TITLE: **Recombinant** SNAP-25 is an effective substrate for **Clostridium botulinum** type A **toxin** endopeptidase activity in vitro
AUTHOR(S): Ekong, Theresa A. N.; Feavers, Ian M.; Sesardic, Dorothea
CORPORATE SOURCE: Division of Bacteriology, National Institute for Biological Standards and Control, Hertfordshire, EN6 3QG, UK
SOURCE: Microbiology (Reading, U. K.) (1997), 143(10), 3337-3347
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bacterial neurotoxins are now being used routinely for the treatment of neuromuscular conditions. Alternative assays to replace or to complement in vivo bioassay methods for assessment of the safety and potency of these botulinum neurotoxin-based therapeutic products are urgently needed. Advances made in understanding the mode of action of clostridial neurotoxins have provided the basis for the development of alternative mechanism-based assay methods. Thus, the identification of SNAP-25 (synaptosomal-assocd. protein of mol. mass 25 kDa) as the intracellular protein target which is selectively cleaved during poisoning by botulinum neurotoxin type A (BoNT/A) has enabled the development of a functional in vitro assay for this toxin. Using recombinant DNA methods, a segment of SNAP-25 (aa residues 134-206) spanning the toxin cleavage site was prepd. as a fusion protein to the maltose-binding protein in Escherichia coli. The fusion protein was purified by affinity chromatog. and the fragment isolated after cleavage with Factor Xa. Targeted antibodies specific for the N and C termini of SNAP-25, as well as the toxin cleavage site, were prepd. and used in an immunoassay to demonstrate BoNT/A endopeptidase activity towards recombinant SNAP-25 substrates. The reaction required

low concns. of reducing agents which were inhibitory at higher concns. as were metal chelators and some inhibitors of metallopeptidases. The endopeptidase assay has proved to be more sensitive than the mouse bioassay for detection of toxin in therapeutic prepns. A good correlation with results obtained in the in vivo bioassay (r = 0.95, n = 23) was demonstrated. The endopeptidase assay described here may provide a suitable replacement assay for the estn. of the potency of type A toxin in therapeutic prepns.

L12 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:358141 CAPLUS
DOCUMENT NUMBER: 127:1856
TITLE: Cleavage of the synaptobrevin/vesicle-associated membrane protein (VAMP) of the mouse brain by the **recombinant** light chain of **Clostridium botulinum** type B **toxin**
AUTHOR(S): Rhee, Sang Dal; Jung, Hyun Ho; Yang, Gi-Hyeok; Moon, Yu Seok; Yang, Kyu-Hwan
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon, S. Korea
SOURCE: FEMS Microbiol. Lett. (1997), 150(2), 203-208
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The light chain of Clostridium botulinum type B toxin was expressed in Escherichia coli using the expression vector pET-3a contg. phage T7 promoter. The expressed protein was then purified by DEAE-cellulose and phosphocellulose chromatog. and the proteolytic activity of the purified light chain was studied. The purified recombinant light chain cleaved synaptobrevin when mixed with the mouse brain microsome and the proteolytic activity of the light chain was inhibited if a metal chelating agent such as EDTA or 2,2'-dipyridyl was added. The recombinant light chain cleaved synaptobrevin more effectively than the native type B toxin. When the native toxin was trypsinized and was reduced with DTT, its proteolytic activity was similar to that of the recombinant light chain.

L12 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:1772 CAPLUS
DOCUMENT NUMBER: 120:1772
TITLE: Similarity in nucleotide sequence of the gene encoding nontoxic component of botulinum toxin produced by toxigenic Clostridium butyricum strain BL6340 and Clostridium botulinum type E strain Mashike
AUTHOR(S): Fujii, Nobuhiro; Kimura, Kouichi; Yokosawa, Noriko; Oguma, Keiji; Yashiki, Teruo; Takeshi, Kouichi; Ohyama, Touru; Isogai, Emiko; Isogai, Hiroshi
CORPORATE SOURCE: Sch. Med., Sapporo Med. Univ., Sapporo, 060, Japan
SOURCE: Microbiol. Immunol. (1993), 37(5), 395-8
CODEN: MIIMDV; ISSN: 0385-5600
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The complete nucleotide and deduced amino acid sequence of the nontoxic component of **botulinum** type E progenitor **toxin** is detd. in **recombinant** plasmid pU9BUH contg. about 6.0 kb HindIII

fragment obtained from chromosomal DNA of **Clostridium** butyricum strain BL6340. The open reading frame (ORF) of this nontoxic component gene is composed of 3,486 nucleotide bases (1,162 amino acid residues). The mol. wt. calcd. from deduced amino acid residues is estd. 13,6810.1. The present study revealed that 33 nucleotide bases of 3,486 are different in the nontoxic component gene between C. butyricum strain BL6340 and C. botulinum type E strain Mashike. This corresponds to the difference of 17 amino acid residues in these nontoxic component.

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=> S (FUSION (5A) PROTEIN (5A) HISTADIN)
        184923 FUSION
          6731 FUSIONS
        188579 FUSION
                 (FUSION OR FUSIONS)
       1301875 PROTEIN
        846090 PROTEINS
       1493884 PROTEIN
                 (PROTEIN OR PROTEINS)
             0 HISTADIN
L1           0 (FUSION (5A) PROTEIN (5A) HISTADIN)

=> S (FUSION (5A) PROTEIN (5A) HISTIDINE)
        184923 FUSION
          6731 FUSIONS
        188579 FUSION
                 (FUSION OR FUSIONS)
       1301875 PROTEIN
        846090 PROTEINS
       1493884 PROTEIN
                 (PROTEIN OR PROTEINS)
         53354 HISTIDINE
          1749 HISTIDINES
         53872 HISTIDINE
                 (HISTIDINE OR HISTIDINES)
L2         216 (FUSION (5A) PROTEIN (5A) HISTIDINE)

=> S L2 AND (L2 AND VACCINE OR IMMUNE COMPOSITION)
         29994 VACCINE
         29247 VACCINES
         37381 VACCINE
                 (VACCINE OR VACCINES)
        122584 IMMUNE
             4 IMMUNES
        122586 IMMUNE
                 (IMMUNE OR IMMUNES)
        559059 COMPOSITION
        219934 COMPOSITIONS
        775536 COMPOSITION
                 (COMPOSITION OR COMPOSITIONS)
       1068630 COMPN
        425024 COMPNS
       1304860 COMPN
                 (COMPN OR COMPNS)
       1716000 COMPOSITION
                 (COMPOSITION OR COMPN)
             7 IMMUNE COMPOSITION
                 (IMMUNE(W)COMPOSITION)
L3          16 L2 AND (L2 AND VACCINE OR IMMUNE COMPOSITION)

=> S L3 AND PY<=1995
      15109814 PY<=1995
L4           4 L3 AND PY<=1995
```

L4 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:693977 CAPLUS
DOCUMENT NUMBER: 121:293977
TITLE: Production in Escherichia coli, purification and immunogenicity of acrosomal protein SP-10, a candidate contraceptive **vaccine**
AUTHOR(S): Reddi, P. Prabhakara; Castillo, James R.; Klotz, Kenneth; Flickinger, Charles J.; Herr, John C.
CORPORATE SOURCE: Center for Recombinant Gamete Contraceptive Vaccinogens, Dept. of Anatomy and Cell Biology, Box 439, University of Virginia, Charlottesville, VA, 22908, USA
SOURCE: Gene (**1994**), 147(2), 189-95
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 3-2 (Biochemical Genetics)
Section cross-reference(s): 13, 15

ABSTRACT:
The testis-specific human sperm antigen, SP-10, has been designated a 'primary ***vaccine*** candidate' by the World Health Organization Taskforce on Contraceptive **Vaccines**. Mol. cloning and sequencing of the cDNAs coding for human (h) and baboon (b) SP-10 have been reported. To produce large amts. of pure antigen for ongoing studies of the immunogenicity and anti-fertility effects of SP-10, we used an efficient Escherichia coli expression system. The full-length open reading frames for hSP-10 and bSP-10 were placed under the inducible T7 bacteriophage RNA polymerase/promoter system. An in-frame fusion was made such that a His6 stretch was produced at the C terminus of SP-10. Upon induction of gene expression, large amts. of hSP-10 or bSP-10 were synthesized and the recombinant (re-) protein segregated into an insol. fraction. The protein was then solubilized in 6 M guanidine.cntdot.HCl and purified by immobilized metal affinity chromatog. (IMAC). The yield of purified bSP-10 prepn. was approx. 20.mu.g/mL of culture.
Immunoreactivity of the purified re-SP-10 with MHS-10, a monoclonal antibody specific to SP-10, and rabbit polyclonal sera raised against SP-10, indicated that the synthesized antigen was suitable for immunization studies. Four female baboons were then immunized with the re-bSP-10 antigen. Immunoblots using pre-immune and immune sera from these animals indicated that all four baboons produced antibodies that reacted with native SP-10 extd. from human sperm in a manner identical to that of MHS-10, the pos. control. Immune sera also stained the acrosome region of human and baboon sperm heads by immunofluorescence. These results demonstrated that the full-length re-bSP-10 antigen was immunogenic in female baboons and generated an immune response which recognized the native antigen on the sperm head, indicating that the recombinant antigen is a suitable **vaccine** immunogen.

SUPPL. TERM: contraceptive **vaccine** acrosomal protein SP10
Escherichia
INDEX TERM: Immunity
(full-length recombinant baboon SP-10 antigen was immunogenic in female baboons and generated an immune response which recognized the native antigen on the sperm head, indicating that the recombinant antigen is a suitable **vaccine** immunogen)
INDEX TERM: Baboon
(immunoblots using pre-immune and immune sera indicated

that all four baboons produced antibodies that reacted with native acrosomal protein SP-10 extd. from human sperm)

INDEX TERM: Antibodies
ROLE: BAC (Biological activity or effector, except adverse);
BIOL (Biological study)
(immunoreactivity of the purified recombinant acrosomal protein SP-10 with rabbit polyclonal sera raised against SP-10 indicated that the synthesized antigen was suitable
for immunization studies)

INDEX TERM: Escherichia coli
**Vaccines**
(prodn. in Escherichia coli, purifn. and immunogenicity of acrosomal protein SP-10, a candidate contraceptive **vaccine**)

INDEX TERM: Antigens
ROLE: BAC (Biological activity or effector, except adverse);
BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process);
USES
(Uses)
(SP-10 (sperm protein 10), prodn. in Escherichia coli, purifn. and immunogenicity of acrosomal protein SP-10, a candidate contraceptive **vaccine**)

INDEX TERM: Virus, bacterial
(T7, human and baboon acrosomal protein SP-10 were placed
under the inducible T7 bacteriophage RNA polymerase/promoter system and produced in Escherichia coli)

INDEX TERM: Sperm
(acrosome, prodn. in Escherichia coli, purifn. and immunogenicity of acrosomal protein SP-10, a candidate contraceptive **vaccine**)

INDEX TERM: Chromatography, column and liquid
(affinity, metal; the human recombinant acrosomal protein
SP-10 produced in Escherichia coli was solubilized in 6 M
guanidine.cntdot.HCl and purified by immobilized metal affinity chromatog.)

INDEX TERM: Gene
ROLE: BAC (Biological activity or effector, except adverse);
BUU (Biological use, unclassified); BIOL (Biological study);
USES (Uses)
(chimeric, an in-frame fusion of human acrosomal protein SP-10 coding sequence was made such that a His6 stretch was produced at the C terminus of SP-10)

INDEX TERM: Antibodies
ROLE: BAC (Biological activity or effector, except adverse);
BIOL (Biological study)
(monoclonal, immunoreactivity of the purified recombinant
acrosomal protein SP-10 with MHS-10, a monoclonal

antibody specific to SP-10, indicated that the synthesized antigen was suitable for immunization studies)

INDEX TERM: Genetic element
ROLE: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(promoter, human and baboon acrosomal protein SP-10 were placed under the inducible T7 bacteriophage RNA polymerase/promoter system and produced in Escherichia coli)

INDEX TERM: 71-00-1, **Histidine**, biological studies
ROLE: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(an in-frame **fusion** of human acrosomal **protein** SP-10 coding sequence was made such that a His6 stretch was produced at the C terminus of SP-10)

INDEX TERM: 9014-24-8, RNA polymerase
ROLE: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(human and baboon acrosomal protein SP-10 were placed under the inducible T7 bacteriophage RNA polymerase/promoter system and produced in Escherichia coli)

L4 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:653576 CAPLUS
DOCUMENT NUMBER: 115:253576
TITLE: A recombinant hybrid protein as antigen for an anti-blood stage malaria **vaccine**
AUTHOR(S): Knapp, B.; Hundt, E.; Enders, B.; Kuepper, H. A.
CORPORATE SOURCE: Res. Lab., Behringwerke A.-G., Marburg, 3550 D, Fed. Rep. Ger.
SOURCE: Behring Inst. Mitt. (**1991**), 88(Mol. Aspects Immunol. Host-Parasite-Interact.), 147-56
CODEN: BHIMA2; ISSN: 0301-0457
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 15-2 (Immunochemistry)
ABSTRACT:
Based on investigations on several blood stage antigens from Plasmodium falciparum, the authors expressed a hybrid protein in Escherichia coli contg. 262 amino acids of the serine-stretch protein SERP and 189 amino acids of the histidine alanine rich protein HRPII. Antibodies raised against the hybrid protein by immunization of rabbits and monkeys react with both corresponding schizont polypeptides. Two monkeys immunized with the SERP/HRPII hybrid protein showed only low parasitemias after challenge infection with P. falciparum, compared to the control group. The result suggests that hybrid proteins of this type may be the basis for the development of a malaria ***vaccine*** .

SUPPL. TERM: malaria chimeric protein **vaccine**
INDEX TERM: **Vaccines**
(for malaria, recombinant hybrid protein as antigen for)

INDEX TERM: Plasmodium falciparum
(hybrid antigen of, infection inhibition by, **vaccine** in relation to)

INDEX TERM: Malaria
(**vaccine** for, recombinant hybrid protein as antigen for)

INDEX TERM: Glycoproteins, specific or class

ROLE: BIOL (Biological study)
(HRPII (**histidine**-rich **protein** II), **fusion** products, with SERA antigen of Plasmodium falciparum, infection inhibition by, **vaccine** in relation to)

INDEX TERM: Antigens
ROLE: BIOL (Biological study)
(SERA (serine-repeat antigen), fusion products, with HRPII protein of Plasmodium falciparum, infection inhibition by, **vaccine** in relation to)

L4 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1990:71651 CAPLUS
DOCUMENT NUMBER: 112:71651
TITLE: Cloning and expression of genetically stable malaria merozoite antigen genes for use as anti-malaria **vaccines**
INVENTOR(S): Certa, Ulrich
PATENT ASSIGNEE(S): Hoffmann-La Roche, F., und Co. A.-G., Switz.
SOURCE: Eur. Pat. Appl., 65 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
INT. PATENT CLASSIF.:
MAIN: C12N015-00
SECONDARY: C07K013-00; C07H021-04; C12N001-20; C12P021-00; A61K039-015; A61K037-02
CLASSIFICATION: 3-4 (Biochemical Genetics)
Section cross-reference(s): 15, 63
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE | |
|---|---|---|---|---|---|
| EP 309746 | A1 | 19890405 | EP 1988-114016 | 19880827 | <-- |
| R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL, SE | | | | | |
| US 5061788 | A | 19911029 | US 1988-237126 | 19880829 | <-- |
| ZA 8806521 | A | 19890426 | ZA 1988-6521 | 19880901 | <-- |
| DK 8804925 | A | 19890309 | DK 1988-4925 | 19880905 | <-- |
| AU 8821877 | A1 | 19890615 | AU 1988-21877 | 19880905 | <-- |
| AU 609183 | B2 | 19910426 | | | |
| JP 01100200 | A2 | 19890418 | JP 1988-222595 | 19880907 | <-- |
| US 5225534 | A | 19930706 | US 1991-737126 | 19910729 | <-- |

PRIORITY APPLN. INFO.: CH 1987-3486 19870908
US 1988-237126 19880829
OTHER SOURCE(S): MARPAT 112:71651
ABSTRACT:
Sequences encoding stable antigen genes from the merozoite stage of Plasmodium falciparum are cloned and expressed in Escherichia coli as **fusion** ***proteins*** with a **histidine**-rich sequence for rapid purifn. by metal chelate affinity chromatog. These antigens are suitable for use as a malaria **vaccine**. Antigenic sequences were cloned by conventional methods and the coding sequence for one of these (K1) was cloned into an expression vector that generated a fusion product with six N-terminal histidines. The protein was subsequently purified from cells expressing the vector by ion-exchange and metal chelate affinity chromatog. (19 mg from 60 g wet cells). The purified protein reacted with anti-merozoite antibodies in Western blots. Endotoxin content of the sample was <3.1 units/mg protein. The
amino acid sequence showed considerable similarity to aldolases and the protein

had detectable aldolase activity.

SUPPL. TERM: plasmodium merozoite antigen gene cloning; malaria merozoite antigen recombinant **vaccine**

INDEX TERM: **Vaccines**
(against malaria, recombinant Plasmodium falciparum merozoite antigens as)

INDEX TERM: Antibodies
ROLE: BIOL (Biological study)
(against recombinant Plasmodium falciparum merozoite antigens, malaria **vaccines** in relation to)

INDEX TERM: Escherichia coli
(cloning and expression in, of chimeric histidine-rich leader sequence-merozoite antigen gene of Plasmodium falciparum)

INDEX TERM: Gene and Genetic element, microbial
ROLE: BIOL (Biological study)
(for cimeric histidine-rich leader sequence-Plasmodium falciparum merozoite antigen, cloning and expression in Escherichia coli of)

INDEX TERM: Antigens
ROLE: BIOL (Biological study)
(gene for, of Plasmodium falciparum merozoite, cloning and expression in Escherichia coli of)

INDEX TERM: Plasmodium falciparum
(merozoite antigens of, recombinant, as malaria **vaccine**)

INDEX TERM: Molecular cloning
(of chimeric histidine-rich leader sequence-Plasmodium falciparum merozoite antigen gene, in Escherichia coli)

INDEX TERM: Protein sequences
(of merozoite antigen of Plasmodium rfalciparum, complete)

INDEX TERM: Malaria
(**vaccines** against, recombinant Plasmodium falciparum merozoite antigen as)

INDEX TERM: Deoxyribonucleic acid sequences
(antigen PMMSA-specifying, of Plasmodium falciparum, complete)

INDEX TERM: Gene and Genetic element, microbial
ROLE: BIOL (Biological study)
(chimeric, for Plasmodium falciparum merozoite antigen and histidine-rich leader, expression, in Escherichia coli of)

INDEX TERM: Proteins, specific or class
ROLE: BIOL (Biological study)
(fusion products, of Plasmodium falciparum merozoite antigen and histidine-rich leader peptide)

INDEX TERM: Peptides, compounds
ROLE: BIOL (Biological study)
(histidine-rich, fusion products, with Plasmodium falciparum merozoite antigen)

INDEX TERM: Plasmid and Episome
(p8/3, Plasmodium falciparum merozoite antigen gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(pDS78/RBSII,6.times.His, histidine-rich leader sequence gene on, metal chelate affinity chromatog. purifn. of recombinant proteins manuf. from)

INDEX TERM: 125052-49-5, Antigen (Plasmodium falciparum clone pK1-B 41-kilodalton reduced) 125052-50-8, 1-247-Antigen (Plasmodium falciparum clone pK1-B 41-kilodalton reduced)
ROLE: PRP (Properties)
(amino acid sequence and expression in Escherichia coli of gene for)

INDEX TERM: 125052-51-9 125052-52-0 125052-53-1 125052-54-2 125052-55-3 125052-56-4 125052-57-5
ROLE: PRP (Properties)
(amino acid sequence of)

INDEX TERM: 125052-58-6
ROLE: BAC (Biological activity or effector, except adverse);
PRP (Properties); BIOL (Biological study)
(amino acid sequence of and expression in Escherichia coli of gene for)

INDEX TERM: 125053-11-4, Deoxyribonucleic acid (Plasmodium falciparum clone pK1-B 41-kilodalton antigen gene) 125053-12-5 125053-14-7, Deoxyribonucleic acid (Plasmodium falciparum clone pK1-B 1-247-41-kilodalton antigen-specifying) 125053-16-9 125053-17-0
ROLE: PRP (Properties); BIOL (Biological study)
(nucleotide sequence and expression in Escherichia coli of)

INDEX TERM: 125053-09-0, Deoxyribonucleic acid (plasmid p8/3) 125053-15-8, Deoxyribonucleic acid (Plasmodium falciparum clone pK1-B 103-362-41-kilodalton antigen-specifying) 125267-86-9
ROLE: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of)

INDEX TERM: 125053-13-6
ROLE: PRP (Properties)
(nucleotide sequence of and expression in Escherichia coli of)

L4 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:187341 CAPLUS
DOCUMENT NUMBER: 110:187341
TITLE: Recombinant preparation of **fusion proteins** containing sequential **histidine** residues, and purification of the proteins by metal chelate affinity chromatography
INVENTOR(S): Doebeli, Heinz; Eggimann, Bernhard; Gentz, Reiner; Hochuli, Erich; Stueber, Dietrich
PATENT ASSIGNEE(S): Hoffmann-La Roche, F., und Co. A.-G., Switz.
SOURCE: Eur. Pat. Appl., 80 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
INT. PATENT CLASSIF.:
MAIN: C07K013-00
SECONDARY: C07K003-18; C12P021-02; C12N015-00; C07K015-26; C12N009-02; C12P021-06; A61K037-02
CLASSIFICATION: 3-5 (Biochemical Genetics)
Section cross-reference(s): 16
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| EP 282042 | A2 | 19880914 | EP 1988-103740 | 19880309 <-- |
| EP 282042 | A3 | 19910911 | | |
| EP 282042 | B1 | 19940608 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| DK 8800842 | A | 19880911 | DK 1988-842 | 19880218 <-- |
| US 5284933 | A | 19940208 | US 1988-158962 | 19880222 <-- |
| ZA 8801534 | A | 19881026 | ZA 1988-1534 | 19880303 <-- |
| AU 8812709 | A1 | 19880915 | AU 1988-12709 | 19880304 <-- |
| AU 609783 | B2 | 19910509 | | |
| AT 106897 | E | 19940615 | AT 1988-103740 | 19880309 <-- |
| JP 63251095 | A2 | 19881018 | JP 1988-55085 | 19880310 <-- |
| US 5310663 | A | 19940510 | US 1993-80043 | 19930618 <-- |
| PRIORITY APPLN. INFO.: | | | CH 1987-895 | 19870310 |
| | | | US 1988-158962 | 19880222 |
| | | | EP 1988-103740 | 19880309 |

ABSTRACT:
Fusion proteins comprising 1 or 2 affinity peptides contg. sequential histidine residues attached directly or indirectly to a biol. active protein are prepd. by recombinant methods. These fusion proteins are purified using a metal-chelating affinity resin with the structure resin-spacer-NH-(CH2)x-CH(COOH)-N-(CH2COO-)2Ni2+. Plasmid pHis,His-Xa-IFN-.gamma., contg. a gene encoding Met-His-His-Ala-Gly-Ile-Glu-Gly-Arg-interferon-.gamma., was constructed. The chimeric gene was expressed in Escherichia coli M15. The fusion protein was purified from a crude lysate of this transformant using a metal-chelating deriv. of Sepharose CL-6B, i.e. [Sepharose CL-6B]-O-CH2-CH(OH)-Cl2)4-CH(COOH)-N-(CH2COO-)2Ni2+. The protein was >90% pure after this treatment.

| | |
|---|---|
| SUPPL. TERM: | fusion protein recombinant purifn; affinity chromatog metal chelate protein purifn |
| INDEX TERM: | Escherichia coli<br>(cloning and expression in, of affinity peptide-biol. active peptide fusion protein gene, metal ion-chelating affinity resin purifn. in relation to) |
| INDEX TERM: | Interferons<br>ROLE: BIOL (Biological study)<br>(fusion products with affinity peptide contg. sequential histidines, recombinant manuf. and purifn. of, with metal ion-chelating affinity resin) |
| INDEX TERM: | **Vaccines**<br>(fusion protein in, recombinant manuf. and purifn. of) |
| INDEX TERM: | Molecular cloning<br>(of biol. active **protein**-sequential **histidine**-contg. affinity peptide **fusion protein** genes, in Escherichia coli) |
| INDEX TERM: | Protein sequences<br>(of interferon-.gamma.-affinity peptide fusion proteins, of human, complete) |
| INDEX TERM: | Proteins, preparation<br>ROLE: PUR (Purification or recovery); PREP (Preparation)<br>(purifn. of, affinity peptide-contg. fusion proteins for, metal ion-chelating affinity chromatog. in) |
| INDEX TERM: | Deoxyribonucleic acid sequences<br>(affinity peptide-interferon .gamma. fusion protein-specifying, of human, complete) |
| INDEX TERM: | Gene and Genetic element |

ROLE: BIOL (Biological study)
(chimeric, for biol. active peptide and affinity peptide,
expression in bacteria of, metal ion-chelating resin purifn. in relation to)

INDEX TERM: **Proteins**, specific or class
ROLE: BIOL (Biological study)
(**fusion** products, affinity peptide-contg., sequential **histidine**-contg., recombinant manuf. and purifn. of, with metal ion-chelating affinity resin)

INDEX TERM: Plasmid and Episome
(p4xHis-DHFR, dihydrofolate reductase-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(p4xHis-DHFR-4xHis, dihydrofolate reductase-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(p6xHis-DHFR, dihydrofolate reductase-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(pDHFR-2xHis, dihydrofolate reductase-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(pDHFR-6xHis, dihydrofolate reductase-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(pHis,His-Ek-IFN-.gamma.(-8), interferon-.gamma. of human-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(pHis,His-Xa-IFN-.gamma., interferon-.gamma. of human-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Plasmid and Episome
(pHis,His-Xa-IFN-.gamma.(-8)(Asn), interferon-.gamma. of human-**histidine**-contg. affinity peptide **fusion protein** gene on, expression in Escherichia coli of)

INDEX TERM: Interferons
ROLE: BIOL (Biological study)
(.gamma., fusion products with sequential histidine-contg. affinity peptide, recombinant manuf. and purifn. of, metal ion-chelating affinity resin in)

INDEX TERM: 120366-76-9 120366-77-0 120366-78-1
ROLE: BAC (Biological activity or effector, except adverse);
PRP (Properties); BIOL (Biological study)
(amino acid sequence of and expression in Escherichia coli of gene for)

INDEX TERM: 9001-92-7, Protease 9002-05-5, Factor Xa
ROLE: PRP (Properties)

(biol. active protein manuf. with, from affinity peptide-contg. fusion protein, metal ion-chelating affinity chromatog. in relation to)

INDEX TERM: 120366-36-1
ROLE: PRP (Properties); BIOL (Biological study)
(cloning and expression in Escherichia coli and nucleotide sequence of)

INDEX TERM: 120366-43-0
ROLE: PRP (Properties); BIOL (Biological study)
(expression in Escherichia coli and nucleotide sequence of)

INDEX TERM: 98059-19-9
ROLE: PRP (Properties)
(expression in Escherichia coli of gene for)

INDEX TERM: 120221-25-2 120221-26-3 120221-27-4 120221-28-5
120221-29-6 120221-30-9 120253-97-6
ROLE: PRP (Properties)
(fusion protein contg., recombinant manuf. and purifn. of, with metal ion-chelating affinity resin)

INDEX TERM: 7440-02-0D, Nickel, complex with lysine, N.alpha.-bis(carboxy methyl), N.epsilon.-(2,3-dihydroxy propyl) 120221-31-0D, nickel complex, Sepharose Cl-6B resin-bound
ROLE: PRP (Properties)
(**fusion protein** purifn. with, sequential **histidine**-contg. affinity peptide-contg., recombinant)

INDEX TERM: 62610-50-8D, Sepharose CL-6B, metal ion-chelating deriv.
ROLE: PRP (Properties)
(**fusion protein** purifn. with, sequential **histidine**-contg., recombinant)

INDEX TERM: 14701-22-5, Nickel(2+), biological studies
ROLE: BIOL (Biological study)
(metal ion-chelating affinity resin contg., recombinant fusion protein purifn. with)

INDEX TERM: 120366-41-8 120366-42-9
ROLE: PRP (Properties); BIOL (Biological study)
(nucleotide sequence and expression in Escherichia coli of)

INDEX TERM: 9002-03-3DP, Dihydrofolate reductase, fusion products with affinity peptide contg. sequential histidines
ROLE: PREP (Preparation)
(recombinant manuf. and purifn. of, with metal ion-chelating affinity resin)

L11 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:227474 CAPLUS
DOCUMENT NUMBER: 135:97336
TITLE: Recombinant derivatives of clostridial neurotoxins as delivery vehicles for proteins and small organic molecules
AUTHOR(S): Zdanovskaia, Marina V.; Los, Georgyi; Zdanovsky, Alexey G.
CORPORATE SOURCE: Promega Corporation, Madison, WI, 53711-5399, USA
SOURCE: J. Protein Chem. (2000), 19(8), 699-707
CODEN: JPCHD2; ISSN: 0277-8033
PUBLISHER: Kluwer Academic/Plenum Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Clostridial neurotoxins are the most powerful toxins known. Nevertheless, derivs. of these toxins may find broad applications both in science and medicine because of their unique abilities to recognize neurons and deliver small and large mols. into them. In this paper we describe the construction of two types of such derivs. Proteins belonging to the first class were designed to allow direct conjugation with one or few mols. of interest. Proteins belonging to the second class contain biotin residues and therefore could be easily connected to streptavidin loaded with multiple mols. of interest. Only C-terminal regions of neurotoxin heavy chains were incorporated in the structure of recombinant proteins. Nevertheless, recombinant proteins were found to be able to recognize specific neuronal receptors and target model mols. to rat synaptosomes and human neuroblastoma cells.
REFERENCE COUNT: 27
REFERENCE(S): (1) Binz, T; Nucleic Acids Res 1990, V18, P5556 CAPLUS
(2) Bizzini, B; Brain Res 1981, V210, P291 CAPLUS
(4) Dunkley, P; Brain Res 1988, V441, P59 CAPLUS
(6) Eisel, U; EMBO J 1986, V5, P2495 CAPLUS
(7) Figueiredo, D; Exp Neurol 1997, V145, P546 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:526203 CAPLUS
DOCUMENT NUMBER: 134:264774
TITLE: Cloning, expression and evaluation of a **recombinant** sub-unit vaccine against **Clostridium botulinum** type F **toxin**
AUTHOR(S): Holley, J. L.; Elmore, M.; Mauchline, M.; Minton, N.; Titball, R. W.
CORPORATE SOURCE: CBD Porton Down, Defence Evaluation and Research Agency, Salisbury, Wilts, SP4 0JQ, UK
SOURCE: Vaccine (2000), 19(2-3), 288-297
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A synthetic gene encoding the Hc (binding) domain of Clostridium botulinum neurotoxin F (FHc) was expressed in Escherichia coli fused to maltose binding protein (MBP). The purified MBP-FHc and FHc isolated after removal of MBP were evaluated in mice for their ability to protect against

toxin challenge. Balb/c mice developed a protective immune response following administration of either protein via the i.p. or i.m. routes. A comparison of antibody titers and protection following single and multiple vaccinations and the effects of dosage are shown. The long term protection afforded by the vaccines was also investigated. Ten months following vaccination mice were still protected when challenged with 104 MLD50 doses of botulinum toxin F.

REFERENCE COUNT: 18

REFERENCE(S):
(4) Byrne, M; Infect Immun 1998, V66, P4817 CAPLUS
(5) Chambers, S; Gene 1988, V68, P139 CAPLUS
(6) Chen, F; Infect Immun 1997, V65, P1626 CAPLUS
(7) Clare, J; Biotechnology 1991, V9, P455 CAPLUS
(8) Clayton, M; Infect Immun 1995, V63, P2738 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:425504 CAPLUS
DOCUMENT NUMBER: 131:72729
TITLE: Vaccine for Clostridium botulinum neurotoxin
INVENTOR(S): Williams, James A.
PATENT ASSIGNEE(S): Ophidian Pharmaceuticals, Inc., USA
SOURCE: U.S., 140 pp., Cont.-in-part of U.S. Ser. No. 329,154, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 9
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 5919665 | A | 19990706 | US 1995-405496 | 19950316 |
| US 5196193 | A | 19930323 | US 1989-429791 | 19891031 |
| US 5601823 | A | 19970211 | US 1993-161907 | 19931202 |
| US 5599539 | A | 19970204 | US 1994-255009 | 19940607 |
| US 5443976 | A | 19950822 | US 1994-275304 | 19940714 |
| US 5904922 | A | 19990518 | US 1995-442000 | 19950516 |
| US 5736139 | A | 19980407 | US 1995-480604 | 19950607 |
| CA 2203504 | AA | 19960502 | CA 1995-2203504 | 19951023 |
| WO 9612802 | A1 | 19960502 | WO 1995-US13737 | 19951023 |
| W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| AU 9539683 | A1 | 19960515 | AU 1995-39683 | 19951023 |
| AU 709586 | B2 | 19990902 | | |
| EP 796326 | A1 | 19970924 | EP 1995-937626 | 19951023 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| BR 9509903 | A | 19971125 | BR 1995-9903 | 19951023 |
| CN 1176658 | A | 19980318 | CN 1995-196424 | 19951023 |
| HU 78048 | A2 | 19990728 | HU 1999-1238 | 19951023 |
| EP 1041149 | A2 | 20001004 | EP 2000-105371 | 19951023 |
| EP 1041149 | A3 | 20010502 | | |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV

| | | | | | |
|---|---|---|---|---|---|
| ZA 9508990 | A | 19960515 | ZA 1995-8990 | | 19951024 |
| FI 9701732 | A | 19970623 | FI 1997-1732 | | 19970423 |
| NO 9701868 | A | 19970624 | NO 1997-1868 | | 19970423 |
| AU 9948763 | A1 | 19991125 | AU 1999-48763 | | 19990916 |
| PRIORITY APPLN. INFO.: | | | US 1989-429791 | A2 | 19891031 |
| | | | US 1992-985321 | A2 | 19921204 |
| | | | US 1993-161907 | A2 | 19931202 |
| | | | US 1994-329154 | B2 | 19941024 |
| | | | US 1992-842709 | A2 | 19920226 |
| | | | US 1992-983668 | B1 | 19921201 |
| | | | US 1994-275304 | A3 | 19940714 |
| | | | US 1995-405496 | A2 | 19950316 |
| | | | US 1995-422711 | A2 | 19950414 |
| | | | US 1995-480604 | A | 19950607 |
| | | | AU 1995-39683 | A3 | 19951023 |
| | | | EP 1995-937626 | A3 | 19951023 |
| | | | WO 1995-US13737 | W | 19951023 |

AB The present invention includes **recombinant** proteins derived from **toxins** of **Clostridium botulinum** and Clostridium difficile. In particular, sol. **recombinant** fusion proteins comprising **Clostridium botulinum** type A **toxin** proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The sol., endotoxin-free recombinant proteins are used as immunogens for the prodn. of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.

REFERENCE COUNT: 117

REFERENCE(S):
(1) Afrin; Bioconj Chem 1994, V5, P539 CAPLUS
(12) Barroso; Nucl Acids Res 1990, V18, P4004 CAPLUS
(13) Beitle; Biotechnol Prog 1993, V9, P64 CAPLUS
(15) Benson, H; J Immunol 1961, V87, P616 CAPLUS
(21) Carroll; US 5196193 1993 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:163478 CAPLUS
DOCUMENT NUMBER: 128:242882
TITLE: Multivalent vaccine for Clostridium botulinum neurotoxin
INVENTOR(S): Williams, James A.; Thalley, Bruce S.
PATENT ASSIGNEE(S): Ophidian Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 428 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9808540 | A1 | 19980305 | WO 1997-US15394 | 19970828 |
| W: AU, CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| AU 9742450 | A1 | 19980319 | AU 1997-42450 | 19970828 |
| EP 1105153 | A1 | 20010613 | EP 1997-940746 | 19970828 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PRIORITY APPLN. INFO.: US 1996-704159 A 19960828
WO 1997-US15394 W 19970828

AB The present invention includes **recombinant** proteins derived from **Clostridium botulinum toxins**. In particular, sol. recombinant Clostridium botulinum type A, type B and type E toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The sol., endotoxin-free recombinant proteins are used as immunogens for the prodn. of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin. Thus, recombinant C. difficile toxin A and B gene and proteins and C. botulinum type A.apprx.G neurotoxin gene and proteins were prepd. as vaccines.

L11 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:358141 CAPLUS
DOCUMENT NUMBER: 127:1856
TITLE: Cleavage of the synaptobrevin/vesicle-associated membrane protein (VAMP) of the mouse brain by the **recombinant** light chain of **Clostridium botulinum** type B **toxin**
AUTHOR(S): Rhee, Sang Dal; Jung, Hyun Ho; Yang, Gi-Hyeok; Moon, Yu Seok; Yang, Kyu-Hwan
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon, S. Korea
SOURCE: FEMS Microbiol. Lett. (1997), 150(2), 203-208
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The light chain of Clostridium botulinum type B toxin was expressed in Escherichia coli using the expression vector pET-3a contg. phage T7 promoter. The expressed protein was then purified by DEAE-cellulose and phosphocellulose chromatog. and the proteolytic activity of the purified light chain was studied. The purified recombinant light chain cleaved synaptobrevin when mixed with the mouse brain microsome and the proteolytic activity of the light chain was inhibited if a metal chelating agent such as EDTA or 2,2'-dipyridyl was added. The recombinant light chain cleaved synaptobrevin more effectively than the native type B toxin. When the native toxin was trypsinized and was reduced with DTT, its proteolytic activity was similar to that of the recombinant light chain.

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| NEWS 4 | Aug 08 | PHARMAMarketLetter(PHARMAML) - new on STN |
| NEWS 5 | Aug 19 | Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN |
| NEWS 6 | Aug 26 | Sequence searching in REGISTRY enhanced |
| NEWS 7 | Sep 03 | JAPIO has been reloaded and enhanced |
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| NEWS 17 | Dec 17 | TOXCENTER enhanced with additional content |
| NEWS 18 | Dec 17 | Adis Clinical Trials Insight now available on STN |
| NEWS 19 | Jan 29 | Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC |
| NEWS 20 | Feb 13 | CANCERLIT is no longer being updated |
| NEWS 21 | Feb 24 | METADEX enhancements |
| NEWS 22 | Feb 24 | PCTGEN now available on STN |
| NEWS 23 | Feb 24 | TEMA now available on STN |
| NEWS 24 | Feb 26 | NTIS now allows simultaneous left and right truncation |
| NEWS 25 | Feb 26 | PCTFULL now contains images |
| NEWS 26 | Mar 04 | SDI PACKAGE for monthly delivery of multifile SDI results |
| NEWS 27 | Mar 20 | EVENTLINE will be removed from STN |
| NEWS 28 | Mar 24 | PATDPAFULL now available on STN |
| NEWS 29 | Mar 24 | Additional information for trade-named substances without structures available in REGISTRY |
| NEWS 30 | Apr 11 | Display formats in DGENE enhanced |
| NEWS 31 | Apr 14 | MEDLINE Reload |
| NEWS 32 | Apr 17 | Polymer searching in REGISTRY enhanced |
| NEWS 33 | Jun 13 | Indexing from 1947 to 1956 added to records in CA/CAPLUS |
| NEWS 34 | Apr 21 | New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX |
| NEWS 35 | Apr 28 | RDISCLOSURE now available on STN |
| NEWS 36 | May 05 | Pharmacokinetic information and systematic chemical names added to PHAR |
| NEWS 37 | May 15 | MEDLINE file segment of TOXCENTER reloaded |
| NEWS 38 | May 15 | Supporter information for ENCOMPPAT and ENCOMPLIT updated |
| NEWS 39 | May 16 | CHEMREACT will be removed from STN |
| NEWS 40 | May 19 | Simultaneous left and right truncation added to WSCA |
| NEWS 41 | May 19 | RAPRA enhanced with new search field, simultaneous left and |

right truncation
NEWS 42 Jun 06 Simultaneous left and right truncation added to CBNB
NEWS 43 Jun 06 PASCAL enhanced with additional data
NEWS 44 Jun 20 2003 edition of the FSTA Thesaurus is now available

NEWS EXPRESS April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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FILE COVERS 1907 - 23 Jun 2003 VOL 138 ISS 26
FILE LAST UPDATED: 22 Jun 2003 (20030622/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> T7lac (w) promoter
23 T7LAC
135510 PROMOTER
46458 PROMOTERS
153911 PROMOTER

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                (PROMOTER OR PROMOTERS)
L1          18 T7LAC (W) PROMOTER

=> chaperon (w) protein
           188 CHAPERON
            54 CHAPERONS
           231 CHAPERON
                (CHAPERON OR CHAPERONS)
       1520631 PROTEIN
       1033319 PROTEINS
       1758497 PROTEIN
                (PROTEIN OR PROTEINS)
L2          20 CHAPERON (W) PROTEIN

=> "recombinant botulinum toxin"
        145337 "RECOMBINANT"
          6063 "RECOMBINANTS"
        148677 "RECOMBINANT"
                ("RECOMBINANT" OR "RECOMBINANTS")
          4310 "BOTULINUM"
             1 "BOTULINUMS"
          4310 "BOTULINUM"
                ("BOTULINUM" OR "BOTULINUMS")
         66932 "TOXIN"
         67515 "TOXINS"
        100429 "TOXIN"
                ("TOXIN" OR "TOXINS")
L3           2 "RECOMBINANT BOTULINUM TOXIN"
                ("RECOMBINANT"(W)"BOTULINUM"(W)"TOXIN")

=> L1 and L3
L4           0 L1 AND L3

=> L2 and L3
L5           0 L2 AND L3

=> week (w0 promoter
MISSING OPERATOR 'WEEK (W0'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> "week promoter"
         65812 "WEEK"
         86240 "WEEKS"
        139659 "WEEK"
                ("WEEK" OR "WEEKS")
        135510 "PROMOTER"
         46458 "PROMOTERS"
        153911 "PROMOTER"
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L6           0 "WEEK PROMOTER"
                ("WEEK"(W)"PROMOTER")

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            27 "WEAKS"
        209487 "WEAK"
                ("WEAK" OR "WEAKS")
        135510 "PROMOTER"
         46458 "PROMOTERS"
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        153911 "PROMOTER"
                 ("PROMOTER" OR "PROMOTERS")
L7         255 "WEAK PROMOTER"
                 ("WEAK"(W)"PROMOTER")

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L8           0 L7 AND L3

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        135510 "PROMOTER"
         46458 "PROMOTERS"
        153911 "PROMOTER"
                 ("PROMOTER" OR "PROMOTERS")
          1612 "T7 PROMOTER"
                 ("T7"(W)"PROMOTER")
L9           0 "T7 PROMOTER" AND L3

=> L7 and L2
L10          0 L7 AND L2

=> L7 and L1
L11          0 L7 AND L1

=> L7 and (recombinant protein"
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message appears repeatedly, please notify the Help Desk.  Enter "HELP
STN" for information on contacting the nearest STN Help Desk by
telephone or via SEND in the STNMAIL file.

=> recombinant (w) proteiin and L7
        145337 RECOMBINANT
          6063 RECOMBINANTS
        148677 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
             8 PROTEIIN
             3 PROTEIINS
            11 PROTEIIN
                 (PROTEIIN OR PROTEIINS)
             0 RECOMBINANT (W) PROTEIIN
L12          0 RECOMBINANT (W) PROTEIIN AND L7

=> recombinant (w) protein and L7
        145337 RECOMBINANT
          6063 RECOMBINANTS
        148677 RECOMBINANT
                 (RECOMBINANT OR RECOMBINANTS)
       1520631 PROTEIN
       1033319 PROTEINS
       1758497 PROTEIN
                 (PROTEIN OR PROTEINS)
         13637 RECOMBINANT (W) PROTEIN
L13          1 RECOMBINANT (W) PROTEIN AND L7

=> DIS L13 1 TI

L13  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS
TI   Engineered GFP as a vital reporter in plants
```

=> DIS L13 1 IBIB ABS
THE ESTIMATED COST FOR THIS REQUEST IS 2.42 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L13 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:198590 CAPLUS
DOCUMENT NUMBER: 124:252099
TITLE: Engineered GFP as a vital reporter in plants
AUTHOR(S): Chiu, Wan-ling; Niwa, Yasuo; Zeng, Weike; Hirano, Takanori; Kobayashi, Hirokazu; Sheen, Jen
CORPORATE SOURCE: Massachusetts General Hospital Department Genetics, Harvard Medical School, Boston, MA, 02114, USA
SOURCE: Current Biology (1996), 6(3), 325-30
CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER: Current Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Background:. The green-fluorescent protein (GFP) of the jellyfish Aequorea victoria has recently been used as a universal reporter in a broad range of heterologous living cells and organisms. Although successful in some plant transient expression assays based on strong promoters or high copy no. viral vectors, further improvement of expression efficiency and fluorescent intensity are required for GFP to be useful as a marker in intact plants. Here, we report that an extensively modified GFP is a versatile and sensitive reporter in a variety of living plant cells and a transgenic plants. Results:. We show that a re-engineered GFP gene sequence, with the favored codons of highly expressed human proteins, gives 20-fold higher GFP expression in maize leaf cells than the original jellyfish GFP sequence. When combined with a mutation in the chromophore, the replacement of the serine at position 65 with a threonine, the new GFP sequence gives more than 100-fold brighter fluorescent signals upon excitation with 490 nm (blue) light, and swifter chromophore formation. We also show that this modified GFP has a broad use in various transient expression systems, and allows the easy detection of **weak promoter** activity, visualization of protein targeting into the nucleus and various plastids, and anal. of signal transduction pathways in living single cells and in transgenic plants. Conclusions:. The modified GFP is a simple and economical new tool for the direct visualization of promoter activities with a broad range of strength and cell specificity. It can be used to measure dynamic responses of signal transduction pathways, transfection efficiency, and subcellular localization of chimeric proteins, and should be suitable for many other applications in genetically modified living cells and tissues of higher plants. The data also suggest that the codon usage effect might be universal, allowing the design of **recombinant proteins** with high expression efficiency in evolutionarily distant species such as humans and maize.

=> DIS L3 1- IBIB ABS
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):Y
THE ESTIMATED COST FOR THIS REQUEST IS 4.83 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:275822 CAPLUS
DOCUMENT NUMBER: 136:273218
TITLE: Use of Botulinum toxins for treating muscle injuries
INVENTOR(S): Brooks, Gregory F.; Aoki, Kei Roger
PATENT ASSIGNEE(S): Allergan Sales, Inc., USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002028425 | A2 | 20020411 | WO 2001-US27193 | 20010831 |
| WO 2002028425 | A3 | 20030227 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 6423319 | B1 | 20020723 | US 2000-678189 | 20001004 |
| AU 2001086991 | A5 | 20020415 | AU 2001-86991 | 20010831 |
| US 2002192240 | A1 | 20021219 | US 2002-155925 | 20020523 |

PRIORITY APPLN. INFO.: US 2000-678189 A 20001004
WO 2001-US27193 W 20010831

AB The invention discloses the use of local administration of a neurotoxin, such as a botulinum toxin, to promote healing and/or to reduce the pain assocd. with an injured muscle.

L3 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:730381 CAPLUS
DOCUMENT NUMBER: 128:10998
TITLE: Induction of an immune response by oral administration of **recombinant botulinum toxin**
AUTHOR(S): Kiyatkin, Nikita; Maksymowych, Andrew B.; Simpson, Lance L.
CORPORATE SOURCE: Dep. Medicine & Biochem. & Molecular Pharmacology, Jefferson Medical College, Philadelphia, PA, 19107, USA
SOURCE: Infection and Immunity (1997), 65(11), 4586-4591
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A gene encoding the full-size botulinum neurotoxin serotype C was reconstructed in vector pQE-30 and expressed at high levels in Escherichia coli. Three amino acid mutations (H229.fwdarw.G,E230.fwdarw.T, and H233.fwdarw.N) were generated in the zinc-binding motif, resulting in complete detoxification of the modified recombinant holotoxin. The PCR-amplified wild-type light chain of botulinum neurotoxin serotype C was also expressed in E. coli and used as a control in all expts. Modified

recombinant holotoxin and light chain contained a histidine affinity tag at the amino terminus, which was used for detection and purifn. Recombinant proteins were purified on nickel affinity resin and analyzed by Western blotting with the anti-histidine tag and anti-neurotoxin C antibodies. The results indicated that the 150-kDa mol. of modified recombinant holotoxin and the 50-kDa recombinant light chain were synthesized without degrdn.; however, E. coli did not provide for efficient nicking of modified recombinant toxin. Modified recombinant holotoxin was not toxic to mice, had no effect on nerve-evoked muscle twitch in vitro, and was not able to cleave syntaxin in crude synaptosome prepns. The recombinant light chain was also nontoxic in vivo, had no effect on evoked muscle twitch, but was able to cleave syntaxin. Modified recombinant neurotoxin and light chain were administered to animals either orally or s.c. Both oral administration and s.c. administration of modified recombinant neurotoxin evoked high levels of serum antibodies and protective immunity. Oral administration of recombinant light chain evoked no systemic response, whereas s.c. administration evoked antibody prodn. and immunity.

=> DIS L2 1- IBIB ABS
YOU HAVE REQUESTED DATA FROM 20 ANSWERS - CONTINUE? Y/(N):Y
THE ESTIMATED COST FOR THIS REQUEST IS 48.30 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L2 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:961690 CAPLUS
DOCUMENT NUMBER: 138:51361
TITLE: Quality control mechanism for membrane glycoproteins by endoplasmic reticulum molecular chaperones
AUTHOR(S): Taira, Hideharu; Yamashita, Tetsuro
CORPORATE SOURCE: Faculty of Agriculture, Iwate University, Japan
SOURCE: Kagaku to Seibutsu (2002), 40(12), 832-842
CODEN: KASEAA; ISSN: 0453-073X
PUBLISHER: Gakkai Shuppan Senta
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review on the modification of secretory proteins and membrane glycoproteins by the addn. of N-linked oligosaccharide chains, endoplasmic reticulum (ER)-assocd. degrdn. and ER quality control of proteins, structure and functions of ER mol. chaperones (BiP/GRP78, calnexin: CNX, calreticulin: CRT, and ERp57/ER-60), protein folding by CNX and CRT, roles of UDP-glucose:glycoprotein glucosyltransferase in protein folding, substrate recognition mechanisms of CNX and CRT, interactions of Sendai virus membrane proteins with ER mol. chaperones, and functions of individual oligosaccharide chains of F and HN proteins of Sendai virus.

L2 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:949707 CAPLUS
TITLE: TROSY experiment for refinement of backbone .psi. and .phi. by simultaneous measurements of cross-correlated relaxation rates and 3,4JH.alpha.HN coupling constants
AUTHOR(S): Voegeli, Beat; Pervushin, Konstantin

CORPORATE SOURCE: Laboratorium fuer Physikalische Chemie, Swiss Federal Institute of Technology, ETH-Hoenggerberg, Zurich, CH-8093, Switz.
SOURCE: Journal of Biomolecular NMR (2002), 24(4), 291-300
CODEN: JBNME9; ISSN: 0925-2738
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The TROSY principle has been introduced into a HNCA expt., which is designed for measurements of the intraresidual and sequential H.alpha.-C.alpha./HN-N dipole/dipole and H.alpha.-C.alpha./N dipole/CSA cross-correlated relaxation rates. In addn., the new expt. provides values of the 3,4JH.alpha. HN coupling consts. measured in an E.COSY manner. The conformational restraints for the .psi. and .phi. angles are obtained through the use of the cross-correlated relaxation rates together with the Karplus-type dependencies of the coupling consts. Improved signal-to-noise is achieved through preservation of all coherence transfer pathways and application of the TROSY principle. The application of the [15N,13C]-DQ/ZQ-[15N,1H]-TROSY-E.COSY expt. to the 16 kDa apo-form of the E. Coli Heme **Chaperon protein** CcmE is described. Overall good agreement is achieved between .psi. and .phi. angles measured with the new expt. and the av. values detd. from an ensemble of 20 NMR conformers.
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:434485 CAPLUS
DOCUMENT NUMBER: 137:290357
TITLE: Formation of organelle and molecular **chaperon** : **protein** support system prepared by the cells
AUTHOR(S): Endo, Toshiya
CORPORATE SOURCE: Graduate School of Science, Nagoya University, Japan
SOURCE: Iden, Bessatsu (2002), 14(Saibo no Mikurokosumosu), 96-106
CODEN: IDBEEU; ISSN: 1340-7376
PUBLISHER: Shokabo
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review, on mol. chaperones; protein organelle membrane permeation; protein folding in organelles; and role mol. chaperons in maintenance and repair of protein structures in the organelles.

L2 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:83669 CAPLUS
DOCUMENT NUMBER: 136:322989
TITLE: Blocking HSF1 by Dominant-Negative Mutant to Sensitize Tumor Cells to Hyperthermia
AUTHOR(S): Wang, Jin-Hui; Yao, Ming-Zhong; Gu, Jin-Fa; Sun, Lan-Ying; Shen, Yu-Fei; Liu, Xin-Yuan
CORPORATE SOURCE: Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of

Sciences, Shanghai, 200031, Peop. Rep. China
SOURCE: Biochemical and Biophysical Research Communications (2002), 290(5), 1454-1461
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Heat shock protein 70 (HSP70), an antiapoptotic **chaperon protein**, is highly expressed in human breast tumors and renders them resistant to such therapy as hyperthermia. In the present study, we inhibited the expression of HSP70 by blocking the heat shock transcription factor 1 (HSF1) function with its dominant-neg. mutant (mHSF1) in Bcap37 cells, a thermotolerant breast cancer cell line. Here we report that retrovirus-mediated transfer of mHSF1 led to massive cell death of Bcap37 after hyperthermia. mHSF1 sensitized Bcap37 cells to hyperthermia by promoting apoptosis induced by heat shock. We also examd. the efficacy of mHSF1 gene therapy in the nude mouse. mHSF1 transfection led to diminution of tumor growth with hyperthermia therapy. Thus, disrupting HSF1 in combination with hyperthermia may open new possibilities for treatment of cancers that have acquired resistance to heat treatment. (c) 2002 Academic Press.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:704745 CAPLUS
DOCUMENT NUMBER: 135:253494
TITLE: Kit for artificial chaperon
INVENTOR(S): Machida, Sachiko; Hayashi, Kiyoshi
PATENT ASSIGNEE(S): Ministry of Agriculture, Forestry and Fisheries of Japan, National Food Research Institute, Japan; Seibutsu Kei Tokutei Sangyo Gijutsu Kenkyu Suishin Kiko
SOURCE: Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 2001261697 | A2 | 20010926 | JP 2000-71533 | 20000315 |

PRIORITY APPLN. INFO.: JP 2000-71533 20000315

AB A kit for artificial chaperon is provided, which is capable of rewinding a protein for which it is difficult or impossible to take a proper conformation without a help by a mol. chaperon due to its low spontaneous folding ability into a proper conformation within a short time, and furthermore, making it fold as an active form. The kit contains a cyclic carbohydrate, cycloamylose, and a polyoxyethylene-type surfactant or an ionic surfactant. In this method of rewinding a protein into a proper conformation and making it fold as an active form, a substance causing a denatured state to the protein is dild. by adding a specific surfactant to

the denatured protein, and the protein is prevented from the aggregation due to self-assocn. Then, cycloamylose is added to remove the surfactant using its inclusion ability.

L2 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:683342 CAPLUS
DOCUMENT NUMBER: 136:36048
TITLE: Importance of the T cell receptor .alpha.-chain transmembrane distal region for assembly with cognate subunits
AUTHOR(S): Shelton, J. G.; Gulland, S.; Nicolson, K.; Kearse, K. P.; Thomas Backstrom, B.
CORPORATE SOURCE: School of Medicine, Department of Microbiology & Immunology, East Carolina University, Greenville, NC, USA
SOURCE: Molecular Immunology (2001), 38(4), 259-265
CODEN: MOIMD5; ISSN: 0161-5890
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Antigen recognition by .alpha..beta. T lymphocytes is mediated via the multisubunit TCR complex consisting of invariant CD3.gamma.,.delta.,.vepsiln. and .zeta. chains assocd. with clonotypic TCR.alpha. and .beta. mols. Charged amino acids located centrally within the TCR.alpha. transmembrane region are necessary and sufficient for assembly with the CD3.delta..vepsiln. heterodimer. Previously, we have shown that deletion of 6-12 amino acids from the carboxy terminus of the TCR.alpha.-chain dramatically abrogates surface TCR expression, suggesting that the distal portion of the TCR.alpha. transmembrane region contains information that regulates the assembly and/or intracellular transport of TCR complexes. We have examd. in more detail the mol. basis for reduced TCR expression in T cells bearing truncated TCR.alpha. chains. We found that in contrast to wild-type (wt), variant TCR.alpha. proteins missing the last nine C-terminal amino acids did not assoc. with core CD3.gamma.,.delta.,.vepsiln. chains and were not assembled into disulfide-linked .alpha..beta. heterodimers. The stability of newly synthesized wt and variant TCR.alpha. mols. was similar, showing that the abrogated surface TCR expression was not a consequence of impaired protein survival. Nevertheless, truncated TCR.alpha. chains still assembled with the **chaperon protein** calnexin in the endoplasmic reticulum, indicating that the distal portion of the TCR.alpha. transmembrane region is not essential for calnexin interaction. These data document a role for the distal portion of the TCR.alpha. transmembrane region in the assembly of TCR complexes and provide a mol. basis for reduced TCR expression in cells bearing truncated TCR.alpha. chains.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:480853 CAPLUS
DOCUMENT NUMBER: 135:118037
TITLE: Suppression of stress proteins, GRP78, GRP94, calreticulin, and calnexin in liver endoplasmic reticulum of rat treated with a highly toxic coplanar PCB

AUTHOR(S): Yoshioka, Yuko; Ishii, Yuji; Ishida, Takumi; Yamada, Hideyuki; Oguri, Kazuta; Motojima, Kiyoto
CORPORATE SOURCE: Grad. Sch. Pharm. Sci., Kyushu Univ., Fukuoka, 812-8582, Japan
SOURCE: Fukuoka Igaku Zasshi (2001), 92(5), 201-216
CODEN: FKIZA4; ISSN: 0016-254X
PUBLISHER: Fukuoka Igakkai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

AB The present study was addressed on the effect of 3,3',4,4',5-pentachlorobiphenyl (PenCB) to expression of mol. **chaperon proteins**, glucose regulated protein (GRP) 78, GRP94, calreticulin, and calnexin in liver endoplasmic reticulum of rat by treatment with acute exposure. Male Wistar rats received PenCB in corn oil at once a doe of 10 mg/kg i.p., then at 5 days after treatment the microsomes were prepd. Free-fed and pair-fed control groups were given the vehicle. The microsomal proteins were sepd. on SDS-PAGE, transferred to membrane and blotted using antibody towards resp. chaperone proteins. The protein levels of GRP78, GRP94, calreticulin and calnexin were significantly decreased with the acute exposure. In addn., albumin level in the microsomes was also significantly reduced by the PenCB treatment. The transferrin level was significantly higher than pair-fed but not from free-fed group. These chaperone proteins have important physiol. functions against synthesized and/or denatured proteins, which include assembling, folding of proteins. PenCB-treatment may alter the extent of biosynthesis of secretory protein such as albumin through the decreasing levels of chaperone proteins in endoplasmic reticulum. Interestingly, reduced GRP78 protein level by PenCB was not due to decreased mRNA level. Our results suggested that a part of the toxicity of PenCB is assocd. to significant decrease of the chaperone proteins in the endoplasmic reticulum.

L2 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:155081 CAPLUS
DOCUMENT NUMBER: 134:337969
TITLE: Isolation and characterisation of putative adhesins from Helicobacter pylori with affinity for heparan sulphate proteoglycan
AUTHOR(S): Ruiz-Bustos, E.; Ochoa, J. L.; Wadstrom, T.; Ascencio, F.
CORPORATE SOURCE: Department of Marine Pathology, Center for Biological Research, La Paz, 23000, Mex.
SOURCE: Journal of Medical Microbiology (2001), 50(3), 215-222
CODEN: JMMIAV; ISSN: 0022-2615
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A pool of heparan sulfate-binding proteins (HSBPs) from Helicobacter pylori culture supernates was obtained by sequential ammonium sulfate pptn. and affinity chromatog. on heparin-Sepharose. The chromatog. procedure yielded one major fraction that contained proteins with heparan sulfate affinity as revealed by inhibition studies of heparan sulfate binding to H. pylori cells. Preparative iso-elec. focusing, SDS-PAGE and blotting expts., with peroxidase(POD)-labeled heparan sulfate as a probe, indicated the presence of two major extracellular proteins with POD-heparan sulfate affinity. One protein had a mol. mass of 66.2 kDa and

a pI of 5.4, while the second protein had a mol. mass of 71.5 kDa and a pI of 5.0. The N-terminal amino acid sequence of the 71.5-kDa HSBP did not show homol. to any other heparin-binding protein, nor to known proteins of H. pylori, whereas the 66.2-kDa HSBP showed a high homol. to an Escherichia coli **chaperon protein** and equine Hb. A third HSBP was isolated from an outer-membrane protein (OMP) fraction of H. pylori cells with a mol. mass of 47.2 kDa. The amino acid sequence of an internal peptide of the OMP-HSBP did not show homol. to the extracellular HSBP of H. pylori, or to another microbial HSBP.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:100780 CAPLUS
DOCUMENT NUMBER: 134:177337
TITLE: Preparation of recombinant viral antigen coexpressed with **chaperon protein**
INVENTOR(S): Furuya, Masahiro; Togi, Akiko; Doi, Atsushi; Ideno, Akira
PATENT ASSIGNEE(S): Sekisui Chemical Co., Ltd., Japan; Kaiyo Biotechnology Laboratory K. K.
SOURCE: Jpn. Kokai Tokkyo Koho, 37 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 2001033448 | A2 | 20010209 | JP 1999-273202 | 19990927 |

PRIORITY APPLN. INFO.: JP 1998-377105 A 19981228
JP 1999-136335 A 19990517

AB Provided is a method to co-express viral antigen gene-encoding vector and chaperon gene-encoding vector in bacterial or yeast host cells. The produced recombinant viral antigens are highly immunogenic and are useful for diagnosis and therapy of viral infection. The chaperon gene is derived from Mathanococcus thermolithotrophicus. Produced recombinant viral antigens are hepatitis B surface or core antigens, hepatitis C core or E1 antigens, and AIDS virus core proteins p24.

L2 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:84449 CAPLUS
DOCUMENT NUMBER: 134:144369
TITLE: Analysis of proteins relating to fruit body formation of Flammulina veltipes
AUTHOR(S): Oda, Aki; Sen, Kikuo; Kurosawa, Shinichi
CORPORATE SOURCE: The United Grad. Sch. Agric. Sci., Gifu Univ., 1-1 Yanagido, Gifu-shi, Gifu, 501-1112, Japan
SOURCE: Nippon Nogei Kagaku Kaishi (2001), 75(1), 21-28
CODEN: NNKKAA; ISSN: 0002-1407
PUBLISHER: Nippon Nogei Kagakkai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB Fruit body formation of basidiomycetes is the most interesting and dynamic

event in their life cycle. SDS-PAGE anal. of total proteins of F. veltipes showed that 58 and 30 kDa proteins appeared at late stages under both under fruiting and non-fruiting conditions. We compared total proteins of aerial hyphae in vegetative stage with those of fruit bodies by SDS-PAGE. Three proteins with mol. masses of 34, 27, and 17 kDa, were expressed only in the fruit bodies. The 17 kDa protein was purified by CM-32 column chromatog. and SDS-PAGE, and its partial amino acids sequence was analyzed. The N-terminus might be modified because of No PTH amino acid were detected. Alignment of two fragments obtained by trypsin digestion were LYDDVVPK and FADENFQLK, resp. These amino acid sequences were 100% the same as cyclophilin of several other organisms. The 17kDa protein may have a role as an intermediate of the cell signaling system in the process of fruit body formation or as a **chaperon protein** with PPIase activity expressed at low temp.

L2 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:579024 CAPLUS
DOCUMENT NUMBER: 133:292553
TITLE: Protein kinase C .mu. is regulated by the multifunctional **chaperon protein** p32
AUTHOR(S): Storz, Peter; Hausser, Angelika; Link, Gisela; Dedio, Jurgen; Ghebrehiwet, Berhane; Pfizenmaier, Klaus; Johannes, Franz-Josef
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, 70569, Germany
SOURCE: Journal of Biological Chemistry (2000), 275(32), 24601-24607
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We identified the multifunctional **chaperon protein** p32 as a protein kinase C (PKC)-binding protein interacting with PKC.alpha., PKC.xi., PKC.delta., and PKC.mu.. We have analyzed the interaction of PKC.mu. with p32 in detail, and we show here in vivo assocn. of PKC.mu., as revealed from yeast two-hybrid anal., pptn. assays using glutathione S-transferase fusion proteins, and reciprocal coimmunopptn. In SKW 6.4 cells, PKC.mu. is constitutively assocd. with p32 at mitochondrial membranes, evident from colocalization with cytochrome c. p32 interacts with PKC.mu. in a compartment-specific manner, as it can be coimmunopptd. mainly from the particulate and not from the sol. fraction, despite the presence of p32 in both fractions. Although p32 binds to the kinase domain of PKC.mu., it does not serve as a substrate. Interestingly, PKC.mu.-p32 immunocomplexes pptd. from the particulate fraction of two distinct cell lines, SKW 6.4 and 293T, show no detectable substrate phosphorylation. In support of a kinase regulatory function of p32, addn. of p32 to in vitro kinase assays blocked, in a dose-dependent manner, aldolase but not autophosphorylation of PKC.mu., suggesting a steric hindrance of substrate within the kinase domain. Together, these findings identify p32 as a novel, compartment-specific regulator of PKC.mu. kinase activity.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:758119 CAPLUS
DOCUMENT NUMBER: 130:221372
TITLE: Kinetics of expression of heat shock protein (HSP)47 in murine model of bleomycin-induced pulmonary fibrosis
AUTHOR(S): Nakahama, Hajime; Kuribayashi, Yasuzo; Matsuyama, Tomohiro; Sugita, Hiroshi; Moriyama, Toshiki; Nagata, Kazuhiro
CORPORATE SOURCE: Fifth Internal Medicine Department, Hyogo Medical University, Japan
SOURCE: Therapeutic Research (1998), 19(10), 3167-3168
CODEN: THREEL; ISSN: 0289-8020
PUBLISHER: Raifu Saiensu Shuppan K.K.
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB The expression of heat-shock protein (HSP) 47 and .alpha.-smooth muscle actin (.alpha.-SMA), a myofibroblast marker, was investigated in mice with bleomycin (BLM)-induced pulmonary fibrosis. HSP47 is a **chaperon protein** which is important in the synthesis of collagen and is thought to be involved in liver and kidney fibrosis. HCl-BLM 3.76 .mu.g/g wt. was introduced into the trachea of B6C3F1 male mice and the mice were examd. after 1, 3, and 7 days. Collagen fibrosis and the expression of .alpha.-SMA and HSP47 appeared in the tracheal areas 7 days after HCl-BLM exposure. The results suggest that HSP47 may also be involved in pulmonary fibrosis.

L2 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:415448 CAPLUS
DOCUMENT NUMBER: 129:226270
TITLE: Co-expression of chaperon gene secB and human lymphotoxin in Escherichia coli
AUTHOR(S): Zhou, Ying; Zhang, Qing; Yin, Changchuan; Song, Daxin; Chen, Yongqing
CORPORATE SOURCE: Department of Microbiology and Institute of Genetics, Fudan University, Shanghai, 200433, Peop. Rep. China
SOURCE: Shengwu Gongcheng Xuebao (1997), 13(4), 433-436
CODEN: SGXUED; ISSN: 1000-3061
PUBLISHER: Kexue Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB SecB was a 17 kDa cytosolic **chaperon protein** that was required for efficient export of particular protein in Escherichia coli. The SecB gene was cloned into plasmid pAcYc184-SecB, which could be coexisted with plasmids with the ColE1 origin. The plasmid pAcYc184-SecB was then transformed into E. coli harboring a high-expression vector of human lymphotoxin gene. The activity increased by about 50% and the induction time was delayed by measuring the anti-tumor activity in the sol. components of cells.

L2 ANSWER 14 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:86470 CAPLUS
DOCUMENT NUMBER: 128:178672
TITLE: Interaction of apolipoprotein E .epsilon. 4 with other genetic and non-genetic risk factors in late onset

Alzheimer disease: problems facing the investigator
AUTHOR(S): Katzman, R.; Kang, D.; Thomas, R.
CORPORATE SOURCE: Department of Neurosciences and the Alzheimer Disease-Research Center, University of California at San Diego, USA
SOURCE: Neurochemical Research (1998), 23(3), 369-376
CODEN: NEREDZ; ISSN: 0364-3190
PUBLISHER: Plenum Publishing Corp.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 82 refs. The Apolipoprotein E4 allele (Apo-.epsilon.4) is the major susceptibility gene for late onset Alzheimer Disease (AD) but epidemiol. data suggest that the effect of this allele is modified in different individuals by genetic or environmental factors. Age and head injury are major non-genetic factors modifying the Apo-e4 risk. There is conflicting data as to whether alleles of other **chaperon proteins** (such as .alpha.1-antichymotrypsin (ACT)) or Apo-.epsilon.4 receptors (such as the VLDL receptor) modify the Apo-E4 risk for AD. We analyze problems posed by genetic assocn. studies including those of multiple comparisons and selection of controls, the latter problem exacerbated by the wide variations in Apolipoprotein E allele frequencies obsd. in different groups and localities.
REFERENCE COUNT: 83 THERE ARE 83 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:202582 CAPLUS
DOCUMENT NUMBER: 126:236148
TITLE: Origins of organelles in plants and algae as inferred from comparisons of highly conserved chaperone proteins
AUTHOR(S): Arakaki, Adrian K.; Viale, Alejandro M.
CORPORATE SOURCE: Departamento de Microbiologia, Faculted de Ciencias Bioquimicas y Farmaceuticas, Programa Multidisciplinario de Biologia Experimental, Universidad Nacional de Rosario, Rosario, 2000, Argent.
SOURCE: Photosynthesis: From Light to Biosphere, Proceedings of the International Photosynthesis Congress, 10th, Montpellier, Fr., Aug. 20-25, 1995 (1995), Volume 1, 971-974. Editor(s): Mathis, Paul. Kluwer: Dordrecht, Neth.
CODEN: 64DFAW
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The mol. chaperones represent distinct families of essential proteins, ubiquitously distributed among eubacteria, mitochondria, and chloroplasts.
Some of those highly conserved proteins, such as Hsp60 and Hsp70, have also proved to constitute valuable phylogenetic tools. The authors have drawn an evolutionary tree based in these mols., and these inferences support a common origin of all plastids from with in the cyanobacterial lineage.

L2 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:869575 CAPLUS

DOCUMENT NUMBER: 123:250105
TITLE: Preparation of **chaperon protein** -rich cell-free protein biosynthesis system from Escherichia coli
INVENTOR(S): Nishimura, Kunihiro; Kitaoka, Yoshihisa; Niwano, Mitsuru
PATENT ASSIGNEE(S): Kobe Steel Ltd, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 4 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 07194374 | A2 | 19950801 | JP 1993-350305 | 19931229 |

PRIORITY APPLN. INFO.: JP 1993-350305 19931229

AB A cell-free protein biosynthesis system enriched with **chaperon proteins** is prepd. by incubating Escherichia coli at 40-45.degree. for 20-60 min followed by prepg. the cellular ext. The system provides a better protein folding environment. Use of the system for the protein synthesis was exemplified by the synthesis of chloramphenicol acetyltransferase (CAT).

L2 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:361160 CAPLUS
DOCUMENT NUMBER: 122:122751
TITLE: Inhibition of carrageenin-induced rat paw edema by crotapotin, a polypeptide complexed with phospholipase A2
AUTHOR(S): Landucci, Elen C. T.; Antunes, Edson; Donato, Jose L.; Faro, Renato; Hyslop, Stephen; Marangoni, Sergio; Oliveira, Benedito; Cirino, Giuseppe; de Nucci, Gilberto
CORPORATE SOURCE: Dep. Biochem., UNICAMP, Campinas, 13081-970, Brazil
SOURCE: British Journal of Pharmacology (1995), 114(3), 578-83
CODEN: BJPCBM; ISSN: 0007-1188
PUBLISHER: Stockton
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effect of purified crotapotin, a non-toxic non-enzymic **chaperon protein** normally complexed to a phospholipase A2 (PLA2) in South American rattlesnake venom, was studied in the acute inflammatory response induced by carrageenin (1 mg/paw), compd. 48/80 (3 .mu.g/paw) and 5-hydroxytryptamine (5-HT) (3 .mu.g/paw) in the rat hind-paw. The effects of crotapotin on platelet aggregation, mast cell degranulation and eicosanoid release from guinea-pig isolated lung were also investigated. Subplantar co-injection of crotapotin (1 and 10 .mu.g/paw) with carrageenin or injection of crotapotin (10 .mu.g/paw) into the contralateral paw significantly inhibited the carrageenin-induced edema. This inhibition was also obsd. when crotapotin (10-30 .mu.g/paw) was administered either i.p. or orally. Subplantar injection of heated crotapotin (15 min at 60.degree.) failed to inhibit carrageenin-induced edema. Subplantar injection of crotapotin (10 .mu.g/paw) also significantly inhibited the rat paw edema induced by compd. 48/80, but it

did not affect 5-HT-induced edema. In adrenalectomized animals, subplantar injection of crotapotin markedly inhibited the edema induced by carrageenin. The inhibitory effect of crotapotin was also obsd. in rats depleted of histamine and 5-HT stores. Crotapotin (30 .mu.g/paw) had no effect on either the histamine release induced by compd. 48/80 in vitro or on the platelet aggregation induced by both arachidonic acid (1 mM) and platelet activating factor (1 .mu.M) in human platelet-rich plasma. The platelet aggregation and thromboxane B2 (TXB2) release induced by thrombin (100 mu mL-1) in washed human platelets were also not affected by crotapotin. In addn., crotapotin (10 .mu.g/paw) did not affect the release of 6-oxo-prostaglandin F1.alpha. and TXB2 induced by ovalbumin in sensitized guinea-pig isolated lungs. These results indicate that the anti-inflammatory activity of crotapotin is not due to endogenous corticosteroid release or inhibition of cyclo-oxygenase activity. It is possible that crotapotin may interact with extracellular PLA2 generated during the inflammatory process thereby reducing its hydrolytic activity.

L2 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:667839 CAPLUS
DOCUMENT NUMBER: 119:267839
TITLE: Adhesion of Bordetella pertussis to eukaryotic cells requires a time-dependent export and maturation of filamentous hemagglutinin
AUTHOR(S): Arico, Beatrice; Nuti, Sandra; Scarlato, Vincenzo; Rappuoli, Rino
CORPORATE SOURCE: Immunobiol. Res. Inst. Siena, Siena, 53100, Italy
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1993), 90(19), 9204-8
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bordetella pertussis, the human pathogen of whooping cough, when grown at 22.degree.C is nonvirulent and unable to bind eukaryotic cells. In response to a temp. shift to 37.degree.C, the bacterium acquires the ability to bind eukaryotic cells in a time-dependent fashion. By studying in vitro the temp.-induced transition, from the nonvirulent to the virulent state, the authors found that binding to CHO cells is mediated by the Arg-Gly-Asp-contg. domain of filamentous hemagglutinin (FHA), a protein with multiple binding specificities. This protein is synthesized as a 367-kDa polypeptide within 10 min after temp. shift, but requires 2 h before it is detected on the bacterial cell surface and starts to bind CHO cells. Mutations affecting the cell surface export of FHA abolish bacterial adhesion to CHO cells, while mutations in the outer membrane protein pertactin strongly reduce binding. This suggests that multiple **chaperon proteins** are required for a correct function of FHA. Finally, several hours after max. binding efficiency is achieved, the N-terminal 220-kDa portion of FHA that contains the binding regions is cleaved off, possibly to release the bacteria from the bound cells and facilitate spreading. The different forms of FHA may play different roles during bacterial infection.

L2 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:643021 CAPLUS
DOCUMENT NUMBER: 119:243021
TITLE: Kill and cure: the promising future for venom research
AUTHOR(S): Dufton, Mark J.
CORPORATE SOURCE: Dep. Pure Appl. Chem., Univ. Strathclyde, UK
SOURCE: Endeavour (1993), 17(3), 138-40
CODEN: ENDEAS; ISSN: 0160-9327
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 12 refs. on the nature of venoms, toxins as affinity labels for receptor purifn. and characterization, therapeutically useful venom components, venom toxins as guide to the design of peptide and protein drugs, augmentation of the toxic and targeting properties of a protein by adding a **chaperon protein** subunit, use of toxins that resemble proteins normally present in the victim, provision of toxin isoforms, pharmacol. effects, and protein engineering.

L2 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1992:441816 CAPLUS
DOCUMENT NUMBER: 117:41816
TITLE: Coexpression of UmuD' with UmuC suppresses the UV mutagenesis deficiency of groE mutants
AUTHOR(S): Donnelly, Caroline E.; Walker, Graham C.
CORPORATE SOURCE: Dep. Biol., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA
SOURCE: Journal of Bacteriology (1992), 174(10), 3133-9
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The GroE proteins of Escherichia coli are heat shock proteins which have been shown to be mol. **chaperon proteins**. Previous work has shown that the GroE proteins of E. coli are required for UV mutagenesis. This process requires the umuDC genes which are regulated by the SOS regulon. As part of the UV mutagenesis pathway, the product of the umuD gene, UmuD, is posttranslationally cleaved to yield the active form, UmuD'. To investigate what role the groE gene products play in UV mutagenesis, UV mutagenesis was measured in groE+ and groE strains which expressed either the umuDC or umuD'C genes. Expression of umuD' instead of umuD will suppress the nonmutability conferred by the groE mutations. However, cleavage of UmuD to UmuD' is unaffected by mutations at the groE locus. Instead, the presence of UmuD' increased the stability of UmuC in groE strains. In addn., evidence was obtained which indicates that GroEL interacts directly with UmuC.

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L1 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:323278 CAPLUS
TITLE: Relaxed Sugar Donor Selectivity of a Sinorhizobium meliloti Ortholog of the Rhizobium leguminosarum Mannosyl Transferase LpcC. Role of the lipopolysaccharide core in symbiosis of Rhizobiaceae

with plants

AUTHOR(S): Kanipes, Margaret I.; Kalb, Suzanne R.; Cotter, Robert J.; Hozbor, Daniela F.; Lagares, Antonio; Raetz, Christian R. H.

CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA

SOURCE: Journal of Biological Chemistry (2003), 278(18), 16365-16371
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The lpcC gene of Rhizobium leguminosarum and the lpsB gene of Sinorhizobium meliloti encode protein orthologs that are 58% identical over their entire lengths of about 350 amino acid residues. LpcC and LpsB are required for symbiosis with pea and Medicago plants, resp. S. meliloti lpsB complements a mutant of R. leguminosarum defective in lpcC, but the converse does not occur. LpcC encodes a highly selective mannosyl transferase that utilizes GDP-mannose to glycosylate the inner 3-deoxy-D-manno-octulosonic acid (Kdo) residue of the lipopolysaccharide precursor Kdo2-lipid IVA. We now demonstrate that LpsB can also efficiently mannosylate the same acceptor substrate as does LpcC. Unexpectedly, however, the sugar nucleotide selectivity of LpsB is greatly relaxed compared with that of LpcC. Membranes of the wild-type S. meliloti strain 2011 catalyze the glycosylation of Kdo2-[4'-32P]lipid IVA at comparable rates using a diverse set of sugar nucleotides, including GDP-mannose, ADP-mannose, UDP-glucose, and ADP-glucose. This complex pattern of glycosylation is due entirely to LpsB, since membranes of the S. meliloti lpsB mutant 6963 do not glycosylate Kdo2-[4'-32P]lipid IVA in the presence of any of these sugar nucleotides. Expression of lpsB in E. coli using a **T7lac promoter**-driven construct results in the appearance of similar multiple glycosyl transferase activities seen in S. meliloti 2011 membranes. Constructs expressing lpcC display only mannosyl transferase activity. We conclude that LpsB, despite its high degree of similarity to LpcC, is a much more versatile glycosyltransferase, probably accounting for the inability of lpcC to complement S. meliloti lpsB mutants. Our findings have important implications for the regulation of core glycosylation in S. meliloti and other bacteria contg. LpcC orthologs.

L1 ANSWER 2 OF 18 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2003:268226 CAPLUS

TITLE: Cloning, analysis, and expression of the gene for thermostable polyphosphate kinase of Thermus caldophilus GK24 and properties of the recombinant enzyme

AUTHOR(S): Hoe, Hyang-Sook; Lee, Sung-Kyoung; Lee, Dae-Sil; Kwon, Suk-Tae

CORPORATE SOURCE: Department of Genetic Engineering, Sungkyunkwan University, Suwon, 440-746, S. Korea

SOURCE: Journal of Microbiology and Biotechnology (2003), 13(1), 139-145
CODEN: JOMBES; ISSN: 1017-7825

PUBLISHER: Korean Society for Microbiology and Biotechnology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene encoding Thermus caldophilus GK24 polyphosphate kinase (Tca PPK) was cloned and sequenced. The gene contains an open reading frame encoding 608 amino acids with a calcd. mol. mass of 69,850 Da. The deduced amino acid sequence of Tca PPK showed a 40% homol. to Escherichia coli PPK, and 39% to Klebsiella aerogenes PPK. The Tca ppk gene was expressed under the control of the **T7lac promoter** on pET-22b(+) in E. coli and its enzyme was purified about 70-fold with 36% yield, following heating and HiTrap chelating HP column chromatog. The native enzyme was found to have an approx. mol. mass of 580,000 Da and consisted of eight subunits. The optimum pH and temp. of the enzyme were 5.5 and 70.degree.C, resp. A divalent cation was required for the enzyme activity, with Mg2+ being the most effective.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L1 ANSWER 3 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:247428 CAPLUS
TITLE: Origin of the 2-Amino-2-deoxy-gluconate Unit in Rhizobium leguminosarum Lipid A
AUTHOR(S): Que-Gewirth, Nanette L. S.; Karbarz, Mark J.; Kalb, Suzanne R.; Cotter, Robert J.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: Journal of Biological Chemistry (2003), 278(14), 12120-12129
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An unusual feature of the lipid A from the plant endosymbionts Rhizobium etli and Rhizobium leguminosarum is the presence of a proximal sugar unit consisting of a 2-amino-2-deoxy-gluconate moiety in place of glucosamine. An outer membrane oxidase that generates the 2-amino-2-deoxy-gluconate unit from a glucosamine-contg. precursor is present in membranes of R. leguminosarum and R. etli but not in S. meliloti or Escherichia coli. We now report the identification of a hybrid cosmid that directs the overexpression of this activity by screening 1800 lysates of individual colonies of a R. leguminosarum 3841 genomic DNA library in the host strain R. etli CE3. Two cosmids (p1S11D and p1U12G) were identified in this manner and transferred into S. meliloti, in which they also directed the expression of oxidase activity in the absence of any chromosomal background. Subcloning and sequencing of the oxidase gene on a 6.5-kb fragment derived from the ~20-kb insert in p1S11D revealed that the enzyme is encoded by a gene (lpxQ) that specifies a protein of 224 amino acid residues with a putative signal sequence cleavage site at position 28. Heterologous expression of lpxQ using the **T7lac promoter** system in E. coli resulted in the prodn. of catalytically active oxidase that was localized in the outer membrane. A new outer membrane protein of the size expected for LpxQ was present in this construct and was subjected to microsequencing to confirm its identity and the site of signal peptide

cleavage. LpxQ expressed in E. coli generates the same products as seen in R. leguminosarum membranes. LpxQ is dependent on O2 for activity, as demonstrated by inhibition of the reaction under strictly anaerobic conditions. An ortholog of LpxQ is present in the genome of Agrobacterium tumefaciens, as shown by heterologous expression of oxidase activity in E. coli.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:872217 CAPLUS
DOCUMENT NUMBER: 138:270337
TITLE: Evaluation of different promoters and host strains for the high-level expression of collagen-like polymer in Escherichia coli
AUTHOR(S): Yin, Jin; Lin, Ju-hwa; Li, Wen-tyng; Wang, Daniel I. C.
CORPORATE SOURCE: Biotechnology Process Engineering Center, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA
SOURCE: Journal of Biotechnology (2003), 100(3), 181-191
CODEN: JBITD4; ISSN: 0168-1656
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The increased expression of collagen-like polymer, CLP3.1-his which consists of 52 repeating peptide (GAPGAPGSQGAPGLQ), in Escherichia coli was investigated. The effects of three promoters, thermally inducible promoter, T7 promoter and **T7lac promoter**, and three Escherichia coli host strains, BL21, BL21(DE3) and BL21(DE3)[pLysS] which differ in stringency of suppressing basal transcription, were compared. Based on the CLP3.1-his expression level, soly. of CLP3.1-his in cells and basal transcription that occurred in the absence of induction, two expression systems, BL21(DE3) contg. plasmid pJY-2 with **T7lac promoter** and BL21(DE3)[pLysS] contg. plasmid pJY-1 with T7 promoter, were selected. With these two expression systems, CLP3.1-his expression levels greater than 40% (g/g) of total cellular proteins and CLP3.1-his concns. of 0.1-0.2 g l-1 can be achieved by using Luria-Bertani medium in shake flask batch cultures. The CLP3.1-his accumulated in the cells is totally sol. and no basal transcription was found before induction. These two high-level expression systems are promising for use in scale-up prodn.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 5 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:556824 CAPLUS
DOCUMENT NUMBER: 137:290770
TITLE: The Escherichia coli gene encoding the UDP-2,3-diacylglucosamine pyrophosphatase of lipid A biosynthesis

AUTHOR(S): Babinski, Kristen J.; Ribeiro, Anthony A.; Raetz, Christian R. H.

CORPORATE SOURCE: Department of Biochemistry, Department of Radiology, Duke University Medical Center, Durham, NC, 27710, USA

SOURCE: Journal of Biological Chemistry (2002), 277(29), 25937-25946
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB UDP-2,3-diacylglucosamine hydrolase is believed to catalyze the fourth step of lipid A biosynthesis in Escherichia coli. This reaction involves pyrophosphate bond hydrolysis of the precursor UDP-2,3-diacylglucosamine to yield 2,3-diacylglucosamine 1-phosphate and UMP. To identify the gene encoding this hydrolase, E. coli lysates generated with individual .lambda. clones of the ordered Kohara library were assayed for overexpression of the enzyme. The sequence of .lambda. clone 157[6E7], promoting overprodn. of hydrolase activity, was examd. for genes encoding hypothetical proteins of unknown function. The amino acid sequence of one such open reading frame, ybbF, is 50.5% identical to a Haemophilus influenzae hypothetical protein and is also conserved in most other Gram-neg. organisms, but is absent in Gram-positives. Cell exts. prepd. from cells overexpressing ybbF behind the **T7lac promoter** have .apprx.540 times more hydrolase activity than cells with vector alone. YbbF was purified to .apprx.60% homogeneity, and its catalytic properties were examd. Enzymic activity is maximal at pH 8 and is inhibited by 0.01% (or more) Triton X-100. The apparent Km for UDP-2,3-diacylglucosamine is 62 .mu.M. YbbF requires a diacylated substrate and does not cleave CDP-diacylglycerol. 31P NMR studies of the UMP product generated from UDP-2,3-diacylglucosamine in the presence of 40% H2180 show that the enzyme attacks the .alpha.-phosphate group of the UDP moiety. Because ybbF encodes the specific UDP-2,3-diacylglucosamine hydrolase involved in lipid A biosynthesis, it is now designated lpxH.

REFERENCE COUNT: 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:424098 CAPLUS

DOCUMENT NUMBER: 134:146118

TITLE: Over-expression of the His6-.gamma./TNF-.beta. protein and single-step purification

AUTHOR(S): Zhou, Qing; Yu, Jian-Fa; Ma, Zhi-Zhang; Ding, Ren-Rui

CORPORATE SOURCE: College of Life Science, Zhejiang University, Hangzhou, 310012, Peop. Rep. China

SOURCE: Zhejiang Daxue Xuebao, Lixueban (2000), 27(2), 188-192
CODEN: ZDXKF6; ISSN: 1008-9497

PUBLISHER: Zhejiang Daxue Chubanshe

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB The hIFN-.gamma./TNF-.beta. fusion protein (h.gamma.TNF-.beta.) recombinant gene was cloned, expression vector pET28 contg. a **T7lac promoter** was constructed, and the h.gamma.TNF-.beta. fusion protein comprising a six consecutive histidine

residues (His6-tag) at N terminus was produced in E. coli. With IPTG (1mM) induction, the expression vector produced a 32 kDa protein that matches the theor. mol. wt. of the His6-.gamma./TNF-.beta., and the product expressed (as insol. inclusion bodies, IBs) is > 45% of the total bacterial proteins. After cell lysis, the IBs is pelleted by centrifugation, dissolved in 7M urea, then purified by Ni column (Ni2+-sepharose 6B). The purity of the product was more than 96% and the recovery rate was 91%. The purified product was refolded at low temp. (i.e. < 10.degree.C). The cytotoxic activity and antiviral activity of the renatured product are 1.2 .times. 107 .apprx. 2.0 .times. 107u/mgp and 6.6 .times. 105 .apprx. 7.2 .times. 105u/mgp resp.

L1 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:226539 CAPLUS
DOCUMENT NUMBER: 131:68807
TITLE: A new series of pET-derived vectors for high efficiency expression of Pseudomonas exotoxin-based fusion proteins
AUTHOR(S): Matthey, Barbel; Engert, Andreas; Klimka, Alexander; Diehl, Volker; Barth, Stefan
CORPORATE SOURCE: Laboratory of Immunotherapy, Dep. I of Internal Medicine, University Hospital of Cologne, Cologne, 50931, Germany
SOURCE: Gene (1999), 229(1-2), 145-153
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant immunotoxins (rITs) are highly specific anti-tumor agents composed of monoclonal antibody fragments or other specific carriers coupled to plant or bacterial toxins. A major problem in the purifn. of rITs is the low periplasmic yield in currently available expression systems. Thus, the aim of this study was the development of a new bacterial expression system for high-level prodn. of rITs. We constructed a series of pET-based vectors for pelB-directed periplasmic secretion or cytoplasmic prodn. under the control of the **T7lac promoter**. Expression in Escherichia coli BL21 (DE3)pLysS allowed a tightly regulated iso-Pr .beta.-d-thiogalactopyranoside (IPTG) induction of protein synthesis. An enterokinase-cleavable poly-histidine cluster was introduced into this setup for purifn. by affinity chromatog. A major modification resulted from the insertion of a specifically designed multiple cloning site. It contains only rare restriction enzyme recognition sites used for cloning of Ig variable region genes, as well as unique SfiI and NotI restriction sites for directed insertion of single-chain variable fragments (scFv) available from established bacteriophage systems. For this purpose, we deleted two naturally occurring internal SfiI consensus sites in a deletion mutant of Pseudomonas aeruginosa exotoxin A (ETA'). Each single structural element of the new vector (promoter, leader sequence, purifn. tag, scFv sequence, selectable marker, and toxin gene) was flanked by unique restriction sites allowing simple directional substitution. The fidelity of IPTG induction and high-level expression were demonstrated using an anti-CD30 scFv (Ki-4) fused to ETA'. These data confirm a bacterial vector system esp. designed

for efficient periplasmic expression of ETA'-based fusion toxins.
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L1 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:671728 CAPLUS
DOCUMENT NUMBER: 130:33638
TITLE: TolAIII co-overexpression facilitates the recovery of periplasmic recombinant proteins into the growth medium of Escherichia coli
AUTHOR(S): Wan, Eugene W.-M.; Baneyx, Francois
CORPORATE SOURCE: Department of Chemical Engineering, University of Washington, Seattle, WA, 98195, USA
SOURCE: Protein Expression and Purification (1998), 14(1), 13-22
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Overprodn. of the third topol. domain of the transmembrane protein TolA (TolAIII) in the periplasm of Escherichia coli confers a "leaky" phenotype to host cells by disrupting the integrity of the outer membrane and causing periplasmic proteins to leach into the growth medium. To examine the physiol. consequences of TolAIII overexpression in more detail and assess the usefulness of this strategy for the release of periplasmic recombinant proteins into the extracellular fluid, we constructed a ColE1-compatible plasmid encoding a fusion between the ribose binding protein signal sequence and TolAIII under T7lac transcriptional control. About half of the total TolAIII synthesized in IPTG-induced cells aggregated in a precursor form in the cytoplasm. However, the majority of the mature protein was sol. and located in the extracellular fluid. TolAIII-overproducing cultures exhibited only slight growth defects upon entry into stationary phase but underwent extensive lysis when treated with 0.1% (w/v) SDS, and were unable to divide when supplemented with 0.02% SDS. The loss of outer membrane integrity resulted in longterm damage since cell viability was reduced by three orders of magnitude compared to control or uninduced cells. Overexpression of TolAIII did not significantly interfere with the translocation and processing of a plasmid-encoded fusion between the OmpA signal sequence and TEM-.beta.-lactamase but led to the release of most periplasmic proteins and 90% of the active enzyme into the extracellular fluid. Although the total levels of .beta.-lactamase accumulation in TolAIII-overproducing cultures was only 1.5- to 2-fold less than in control cells, the formation of periplasmic inclusions bodies was completely suppressed. A threshold concn. of TolAIII was necessary for efficient release of periplasmic proteins since the viability and detergent sensitivity of uninduced cells was comparable to that of control cultures and 80% of the .beta.-lactamase synthesized remained confined to the periplasm. (c) 1998 Academic Press.
REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L1 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:255044 CAPLUS
DOCUMENT NUMBER: 128:307568
TITLE: Increased expression of Brevibacterium sterolicum cholesterol oxidase in Escherichia coli by genetic modification
AUTHOR(S): Sampson, Nicole S.; Chen, Xiaoyu
CORPORATE SOURCE: Department of Chemistry, State University of New York, Stony Brook, NY, 11794-3400, USA
SOURCE: Protein Expression and Purification (1998), 12(3), 347-352
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To improve expression of Brevibacterium sterolicum cholesterol oxidase in Escherichia coli, we utilized the **T7lac promoter** and modified the gene to encode the first 21 amino acids with high-expression E. coli codons. These changes resulted in a 60-fold improvement of expression level. N-terminal sequencing revealed that the E. coli produced cholesterol oxidase signal peptide is cleaved 6 amino acids closer to the N-terminus than in B. sterolicum. The recombinant E. coli produced protein is composed of 513 amino acids with a calcd. Mr of 55,374. The kinetic rate consts. of the recombinant protein and the B. sterolicum produced cholesterol oxidase are identical.
REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:509895 CAPLUS
DOCUMENT NUMBER: 127:92011
TITLE: Expression of an Aspergillus niger Phytase ( Escherichia coli
AUTHOR(S): Phillippy, Brian Q.; Mullaney, Edward J.
CORPORATE SOURCE: Southern Regional Research Center Agric Research Service, U.S. Department of A Orleans, LA, 70124, USA
SOURCE: Journal of Agricultural and Food C 45(8), 3337-3342
CODEN: JAFCAU; ISSN: 0021-8561
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene (phyA) for the Aspergillus niger phy and 2.2 was expressed in Escherichia coli u **T7lac promoter**. A 56 kDa fusion protein c phytase linked to an S-tag leader peptid at 30.degree.. The yield of unglycosyl from 50 mL cultures by anion exchange body protein was 10 mg. The refold .mu.mol mg-1 min-1 at 37.degree., of inactive aggregates. Recombi optimum at pH 5.1, was irreversibly dena 55.degree.. As with A. niger phyt obsd. was inositol 1,2,4,5,6-pentakis(phosph

of inositol hexakis(phosphate) and p-nitrophenylphosphate were 96 .mu.M and 2.0 mM, resp., at pH 4.5.

L1 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:7211 CAPLUS
DOCUMENT NUMBER: 126:44252
TITLE: High level expression of Ricinus communis casbene synthase in Escherichia coli and characterization of the recombinant enzyme
AUTHOR(S): Hill, Alison M.; Cane, David E.; Mau, Christopher J. D.; West, Charles A.
CORPORATE SOURCE: Dep. Chemistry, Brown Univ., Providence, RI, 02912, USA
SOURCE: Archives of Biochemistry and Biophysics (1996), 336(2), 283-289
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Casbene synthase (I) catalyzes the cyclization of geranylgeranyl diphosphate to casbene, a diterpene phytoalexin with antibacterial and antifungal activity that is produced by seedlings of castor bean (Ricinus communis L.) in response to fungal attack. Here, the authors report the high-level expression of I cDNA in Escherichia coli as insol. inclusion bodies, the solubilization and refolding of active I, and the kinetic and product anal. of recombinant I. To overcome problems apparently assocd. with the presence in the I gene of rare Arg codons, as well as the intrinsic antibacterial activity of casbene itself, the I gene was expressed in a E. coli host harboring the pSM102 vector that encodes the dnaY gene for tArg(AGA/G), using expression vector pET-21d(+) carrying the tightly controlled **T7lac promoter**.

L1 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:272108 CAPLUS
DOCUMENT NUMBER: 124:334289
TITLE: T7 vectors with a modified **T7lac promoter** for expression of proteins in Escherichia coli
AUTHOR(S): Peranen, Johan; Rikkonen, Marja; Hyvoenen, Marko; Kaariainen, Leevi
CORPORATE SOURCE: Inst. Biotechnol., Univ. Helsinki, Helsinki, FIN-00014, Finland
SOURCE: Analytical Biochemistry (1996), 236(2), 371-3
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The prodn. of heterologous proteins in Escherichia coli (E. coli) has become much easier with the introduction of the T7 expression system. However, the expression of toxic proteins to the bacterial cell is hampered due to the leakiness of the system. Different strategies have been developed to overcome this problem. In this study we present the construction and use of new T7lac expression vectors (pBAT, pHAT and pRAT) that allow for the expression of proteins toxic to E. coli.

L1 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:98228 CAPLUS
DOCUMENT NUMBER: 124:224535

TITLE: Galactofuranose biosynthesis in Escherichia coli K-12: identification and cloning of UDP-galactopyranose mutase

AUTHOR(S): Nassau, Pam M.; Martin, Stephen L.; Brown, Robin E.; Weston, Anthony; Monsey, David; McNeil, Michael R.; Duncan, Kenneth

CORPORATE SOURCE: Glaxo Wellcome Medicines Research Center, Hertfordshire, SG1 2NY, UK

SOURCE: Journal of Bacteriology (1996), 178(4), 1047-52
CODEN: JOBAAY; ISSN: 0021-9193

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have cloned two open reading frames (orf6 and orf8) from the Escherichia coli K-12 rfb region. The genes were expressed in E. coli under control of the **T7lac promoter**, producing large quantities of recombinant protein, most of which accumulated in insol. inclusion bodies. Sufficient sol. protein was obtained, however, for use in a radiometric assay designed to detect UDP-galactopyranose mutase activity (the conversion of UDP-galactopyranose to UDP-galactofuranose). The assay is based upon high-pressure liq. chromatog. sepn. of sugar phosphates released from both forms of UDP-galactose by phosphodiesterase treatment. The crude orf6 gene product converted UDP-[.alpha.-D-U-14C]-galactopyranose to a product which upon phosphodiesterase treatment gave a compd. with a retention time identical to that of synthetic .alpha.-galactofuranose-1-phosphate. No mutase activity was detected in exts. from cells lacking the orf6 expression plasmid or from orf8-expressing cells. The orf6 gene product was purified by anion-exchange chromatog. and hydrophobic interaction chromatog. Both the crude ext. and the purified protein converted 6 to 9% of the UDP-galactopyranose to the furanose form. The enzyme was also shown to catalyze the reverse reaction; in this case an approx. 86% furanose-to-pyranose conversion was obsd. These observations strongly suggest that orf6 encodes UDP-galactopyranose mutase (EC 5.4.99.9), and we propose that the gene be designated glf accordingly. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of purified UDP-galactopyranose mutase revealed one major band, and anal. by electrospray mass spectrometry indicated a single major species with a mol. wt. of 42,960 .+-. 8, in accordance with that calcd. for the Glf protein. N-terminal sequencing revealed that the first 15 amino acids of the recombinant protein corresponded to those expected from the published sequence. UV-visible spectra of purified recombinant enzyme indicated that the protein contains a flavin cofactor, which we have identified as FAD.

L1 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1995:492945 CAPLUS

DOCUMENT NUMBER: 122:263611

TITLE: Comparison of the expression of native and mutant bovine annexin IV in Escherichia coli using four different expression systems

AUTHOR(S): Nelson, Michael R.; Creutz, Carl E.

CORPORATE SOURCE: Dep. Pharmacol., Univ. Virginia, Charlottesville, VA, 22908, USA

SOURCE: Protein Expression and Purification (1995), 6(2), 132-40

CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bovine annexin IV, a Ca2+-dependent, membrane-binding protein, was expressed in E. coli using 4 different prokaryotic expression vector systems. An annexin IV cDNA was mutated in the 5' noncoding region to introduce an NcoI restriction site at the translation initiation site. The coding sequence was then excised and ligated into the expression vectors: pKK233-2 (which uses a hybrid trc promoter), pFOG405 (which uses the alk. phosphatase promoter and generates a fusion protein with the alk. phosphatase signal sequence that targets the protein for secretion), pOTSNco12 (which provides temp.-sensitive expression from the .lambda. phage promoter), and pET11d (which uses the **T7lac promoter** and a protease-deficient host). Expression of wild type and mutant annexin IV in the various systems was compared. Differences in level of expression, formation of inclusion bodies, and yield of purified protein were obsd. The pET11d system was the most effective expression system for annexin IV and various annexin IV mutant constructs, providing the highest yield of functional protein from the sol. fraction of cell lysates. Bovine chromaffin granule binding and aggregating activities of recombinant annexin IV were virtually indistinguishable from those of bovine annexin IV isolated from liver tissue. Truncation constructs contg. 1, 2, or 3 of the 4 conserved 70-amino-acid domains of native annexin IV were successfully created and expressed in E. coli, but the recombinant proteins were generally insol. The pET11d annexin constructs contg. point mutations in residues involved in binding Ca produced sol. protein at levels comparable to those of constructs expressing wild type protein.

L1 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:364965 CAPLUS
DOCUMENT NUMBER: 122:257625
TITLE: Sequence, expression and transcriptional analysis of the coronafacate ligase-encoding gene required for coronatine biosynthesis by Pseudomonas syringae
AUTHOR(S): Liyanage, H.; Penfold, C.; Turner, J.; Bender, C. L.
CORPORATE SOURCE: Department of Plant Pathology, Oklahoma State University, Stillwater, OK, 74078-9947, USA
SOURCE: Gene (1995), 153(1), 17-23
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Pseudomonas syringae pv. glycinea PG4180 produces the chlorosis-inducing phytotoxin coronatine (COR), which consists of a polyketide component, coronafacic acid (CFA), ligated by an amide bond to coronamic acid (CMA), an ethylcyclopropyl amino-acid derived from isoleucine. The nucleotide sequence is reported for a 2.37-kb region contg. the coronafacate ligase-encoding gene (cfl) which is required for the amide linkage of CFA and CMA. The transcription start point for cfl was identified, and the Cfl protein was overproduced from the **T7lac promoter** in Escherichia coli. The deduced amino acid sequence of Cfl showed homol. to a variety of adenylate-forming enzymes which bind and hydrolyze ATP in order to activate their substrates for further ligation.

L1 ANSWER 16 OF 18 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1995:6882 CAPLUS
DOCUMENT NUMBER: 122:2127
TITLE: Phagemid pSIT permits efficient in vitro mutagenesis and tightly controlled expression in E. coli
AUTHOR(S): Andreansky, Martin; Hunter, Eric
CORPORATE SOURCE: Univ. Alabama, Birmingham, AL, USA
SOURCE: BioTechniques (1994), 16(4), 626, 628, 630, 632-3
CODEN: BTNQDO; ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A new phagemid vector, pSIT, was constructed that allows both oligonucleotide-directed mutagenesis and tightly controlled, high-level expression of proteins in Escherichia coli. An efficient rate of mutagenesis is achieved by taking advantage of the double oligonucleotide primer technique. In addn. to the mutagenic primer, a second oligonucleotide primer conferring antibiotic resistance to the mutant DNA strand is annealed to single-strand DNA. Selection for the antibiotic thus increases the frequency of mutants. High-level and tightly controlled expression of heterologous proteins is enabled by utilizing a very strong hybrid **T7lac promoter** and lac repressor in conjunction with T7 RNA polymerase as well as a high copy no. of the vector. The pSIT phagemid permits overexpression of proteins and their mutants without having to do subclonings from mutagenic to expression constructs; this saves time, esp. when multiple mutations of the same protein are proposed. A retroviral proteinase precursor, toxic for E. coli, was successfully expressed to a high level, and a series of mutants of this protein was readily obtained.

L1 ANSWER 17 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1994:526294 CAPLUS
DOCUMENT NUMBER: 121:126294
TITLE: The pKSM710 vector cassette provides tightly regulated lac and **T7lac promoters** and strategies for manipulating N-terminal protein sequences
AUTHOR(S): Maneewannakul, Sumit; Maneewannakul, Kesmanee; Ippen-Ihler, Karin
CORPORATE SOURCE: Dep. Med. Microbiol. Immunol., Texas A and M Univ., College Station, TX, 77483, USA
SOURCE: Plasmid (1994), 31(3), 300-307
CODEN: PLSMDX; ISSN: 0147-619X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors describe a set of plasmid vectors that are very useful for cloning, expressing, mutagenizing, deleting, and sequencing DNA fragments. A strategy for using one (pKSM717) to obtain mutant protein products that contain deletions of N-terminal amino acids is also presented. Desirable sequences were first combined in plasmid pKSM710 in a manner that facilitates construction of similar vectors carrying alternative selectable markers or replication origins: a cassette that includes LacI-regulated T7 (T7lac) and lacUV5 promoters, a multiple cloning site (MCS)/lacZ.alpha. sequence, a set of transcription terminators (T.vphi., rrnBT1, rrnBT2, and Tfd), and an fd origin of replication can be moved as a single unit. Alternative restriction sites permit a .lambda.PL promoter and/or the sequence of the pMB1 replicon to be included in this unit when desired. With vectors contg. the cassette, inserts in the MCS can be identified by their lack of lacZ.alpha. peptide complementing activity and

expressed from the dually regulated T7 (T7lac) and/or lacUV5 promoter. The authors found expression from this pair of promoters to be very tightly regulated in appropriate hosts; the degree of repression obtainable in the absence of inducer (IPTG) should allow these constructs to be useful for engineering and expressing gene products that are potentially toxic to the cell. Using the pKSM710 cassette, the authors made derivs. carrying kan (KmR) (pKSM711, pKSM712), kan lacI (pKSM715), kan and lacIq (pKSM713, pKSM714), and amp (pKSM717, pKSM718). One can use pKSM717 to obtain deletion derivs. that lack the original amino-terminal coding region of a cloned gene sequence but express the polypeptide encoded the portion of the gene that remains.

L1 ANSWER 18 OF 18 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1991:402408 CAPLUS
DOCUMENT NUMBER: 115:2408
TITLE: Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with lac repressor
AUTHOR(S): Dubendorff, John W.; Studier, F. William
CORPORATE SOURCE: Biol. Dep., Brookhaven Natl. Lab., Upton, NY, 11973, USA
SOURCE: Journal of Molecular Biology (1991), 219(1), 45-59
CODEN: JMOBAK; ISSN: 0022-2836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Effects of placing a lac operator at different positions relative to a promoter for bacteriophage T7 RNA polymerase were tested. Transcription can be strongly repressed by lac repressor bound to an operator centered 15 base-pairs downstream from the RNA start, but T7 RNA polymerase initiates transcription very actively from this **T7lac promoter**-operator combination in the absence of repressor, or in the presence of repressor plus inducer. Sequence changes in the transcribed region were found to make transcription from some T7 promoters, including the **T7lac promoter**, more sensitive to inhibition by T7 lysozyme. The pET-10 and pET-11 series of plasmid vectors have been constructed to allow target genes to be placed under control of the **T7lac promoter** and to be expressed in BL21(DE3) or HMS174(DE3), which carry an inducible gene for T7 RNA polymerase. These vectors carry a lacI gene that provides enough lac repressor to repress both the **T7lac promoter** in the multicopy vectors and the chromosomal gene for T7 RNA polymerase, which is controlled by the lacUV5 promoter. Very low basal expression of target genes is achieved, but the usual high levels of expression are obtained upon induction. Addn. of T7 lysozyme can reduce basal expression even further and still allow high levels of expression upon induction. Genes that are very toxic to Escherichia coli can be maintained and expressed in this system.

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